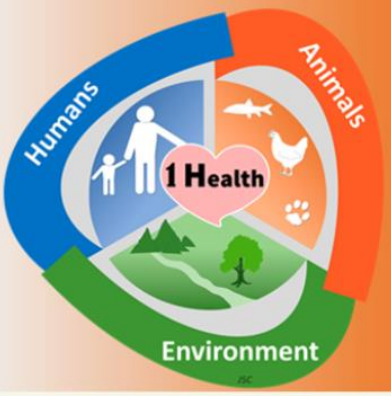
Scientific Letters

VOLUME 1 SUPPLEMENT 1 (2023) ISSN 2795-5117

II TOXRUN INTERNATIONAL CONGRESS 2023



27-28 April, 2023 | Porto, Portugal

BOOK OF ABSTRACTS

IUCS-CESPU Publishing

EDITORIAL OFFICE

IUCS-CESPU Publishing
Rua Central de Gandra, 1317
4585-116, Gandra PRD
Portugal

ADMINISTRATIVE EDITOR

Rui Azevedo

https://doi.org/10.48797/sl.2023.111

For citation purposes, cite each abstract independently as indicated on the abstract page at https://publicacoes.cespu.pt/index.php/sl/issue/view/5.

II TOXRUN INTERNATIONAL CONGRESS 2023

27TH – 28TH OF APRIL
PORTO / PORTUGAL

NO BOUNDARIES FOR TOXICOLOGY:
ONE HEALTH, ONE SOCIETY, ONE PLANET

Authors of abstracts published in the present Book of Abstracts are cordially invited to submit their full manuscripts to *Scientific Letters* for publication. Please note that these manuscripts will undergo peer-review according to the review policy of the journal.

PRESIDENT OF THE ORGANIZING COMMITTEE

Ricardo Jorge Dinis-Oliveira (TOXRUN-IUCS-CESPU; FMUP; UCIBIO-REQUIMTE)

ORGANIZING COMMITTEE

Alexandra Teixeira (TOXRUN-IUCS-CESPU)
Áurea Carvalho (TOXRUN-IUCS-CESPU; LAQV-REQUIMTE)
Daniel Mongiovi (TOXRUN-IUCS-CESPU)
Eduarda Silva (TOXRUN-IUCS-CESPU; LAQV-REQUIMTE)
Joana Barbosa (TOXRUN-IUCS-CESPU; UCIBIO-REQUIMTE)
Rui Azevedo (TOXRUN-IUCS-CESPU)
Sara Ricardo (TOXRUN-IUCS-CESPU; i3S-UP; FMUP)

SCIENTIFIC COMMITTEE

Ana R. Freitas (TOXRUN-IUCS-CESPU; UCIBIO-REQUIMTE; i4HB)
Bruno Peixoto (TOXRUN-IUCS-CESPU; CINTESIS@RISE)
Cláudia Ribeiro (TOXRUN-IUCS-CESPU)
Daniel Barbosa (TOXRUN-IUCS-CESPU; i3S-UP)
Diana Dias da Silva (TOXRUN-IUCS-CESPU; UCIBIO-REQUIMTE; i4HB; ESSLei)
Juliana Faria (TOXRUN-IUCS-CESPU; UCIBIO-REQUIMTE)
Sandra Leal (TOXRUN-IUCS-CESPU; CINTESIS@RISE)

CONGRESS SECRETARIAT

Carla Batista-Pinto (TOXRUN-IUCS-CESPU)
Fernanda Pereira (IUCS-CESPU)
Patrícia Silva (TOXRUN-IUCS-CESPU; UNIPRO-IUCS-CESPU)
Virgínia Gonçalves (TOXRUN-IUCS-CESPU; UNIPRO-IUCS-CESPU)

WEBMASTER

Patrícia Silva
Rui Azevedo
Virgínia Gonçalves

CONGRESS FINANCIAL MANAGEMENT

Ricardo Jorge Dinis-Oliveira
Rui Coelho
Virgínia Gonçalves

VENUE

II TOXRUN International Congress 2023

No Boundaries for Toxicology: One Health, One Society, One Planet

Porto, 27th and 28th April 2023

Hotel Cristal Porto

Organizing Entities

- TOXRUN – Toxicology Research Unit
- IUCS – Instituto Universitário de Ciências da Saúde, CESPU

CONTENTS

EDITORIAL

II TOXRUN International Congress 2023 | No Boundaries for Toxicology: One Health, One Society, One Planet

Ricardo Jorge Dinis-Oliveira [1,2,3,*], Scientific and Organizing Committees [1]

[1] TOXRUN–Toxicology Research Unit, University Institute of Health Sciences (IUCS), CESPU. 4585-116 Gandra, Portugal
[2] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
[3] UCIBIO-REQUIMTE-Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

* Correspondence: ricardo.dinis@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.48

Welcome to the II TOXRUN INTERNATIONAL CONGRESS, 2023 entitled “No Boundaries For Toxicology: One Health, One Society, One Planet. We intend that this event constitutes a space for approximation between professionals who work in life and health sciences and who see Toxicology as a privileged stage for the understanding that there is only “One Health”, as well as an opportunity for technical-scientific updating and debate. Therefore, this welcome Editorial also emphasizes the application of the “One Health” concept to Toxicology and highlights the Mission of TOXRUN for society development.

The interdisciplinary disruptive perspective of modern Toxicology and Pharmacology offers advanced and novel knowledge focused on safety and health risks and on the prevention and mitigation of damages from social- to environment-related pressures on health and wellbeing. A collaborative approach, breaking the virtual illogical boundaries between animal, human, and environmental health, is needed to develop plans for response and control. Particularly, One Health Toxicology, a branch of Toxicology, takes a holistic approach to study the effects of toxins and toxics on human, animal, and environmental health following a multidisciplinary methodology [1]. It recognizes that humans, animals, and the environment are all interconnected and that xenobiotics can have a mutual cascading effect on all three. Particularly, TOXRUN is rapidly evolving in the context of a dynamic scientific environment, with strongly committed researchers endowed with a vast research experience as measured for example by their h-indexes and high rate of publication in prestigious peer-reviewed journals, and with very favorable institutional conditions at all levels of the required administrative/organizational/financial processes. In the One Health perspective, TOXRUN intends to encompass the pharmacological and toxicological study of xenobiotics and endobiotics (e.g., pharmaceuticals, pesticides, psychoactive substances) in a fully integrated perspective, from their genesis to their end, but also opening the door to everything that is deleterious to life and sometimes considered far apart, such as psychological conditions involving stress or anxiety, genetic conditions, nutritional/ lifestyle behaviors, environmental factors, among many others. This integrative perspective maximizes all the different scientific backgrounds of our members and the potential for interesting and disruptive research, which often only occurs in the presence of multidisciplinary teams. To reinforce this idea, 4 research lines were outlined with the main objective of having a real and effective impact in decision making at different levels of the society: TL1 – Biomarkers, Drugs Research and Diagnostics; TL2 – Biopsychosocial Risk Factors and Resilience; TL3 – Toxicological, Food and Environmental Safety; TL4 – Real World Data & Evidence and Decision Making.

The II TOXRUN International Congress 2023 aims to provide an enjoyable and successful environment for professionals working in Toxicology and all biomedical- and environmental-related areas to debate technical-scientific updates and share experiences and reflections. This year's Congress is under the theme “NO BOUNDARIES FOR TOXICOLOGY: ONE HEALTH, ONE SOCIETY, ONE PLANET”. A set of topics that we consider to be timely and of the greatest relevance will be addressed by prestigious personalities who will share their knowledge and research clearly demonstrating the position of Toxicology towards the One Health umbrella. This is a concept that TOXRUN aims to cultivate and obtain scientific outputs. A particularly relevant moment will be the presentation of Poster and Oral Communications on different topics of Toxicology and related areas. After the I TOXRUN International Congress, organized together with Portuguese Association of Forensic Sciences in 2022, this year we decided to jump for the maturity and promote an individual meeting. As proof of TOXRUN vitality, we received 89 submissions,

68 of them were accepted for poster communications and 20 were selected for oral presentations. Our objective was to bring attention to the interdisciplinary perspective of modern Toxicology and to promote awareness and initiatives to build resilient communities and a circular economy. Innovative research directly related to Toxicology (e.g., ecotoxicology, education in toxicology, food toxicology, environmental toxicology, analytical toxicology, clinical toxicology, forensic toxicology, veterinary toxicology, occupational toxicology, toxinology, regulatory toxicology) but also at interface of Biomedical, Biochemistry and Environmental Sciences (e.g., Medicine, Nutrition, Veterinary Medicine, Pharmaceutical Sciences and Forensic Sciences) with cross-cutting issues within the Sustainable Development Goals are within our main focus to increase the strength and recognition of TOXRUN.

In this 2023 congress, we wish to give the first push and discuss Toxicology in a unifying approach to balance and optimize the health of people, animals, and the environment. Finally, and most importantly, I would like to acknowledge the Organizing and Scientific committees for their outstanding collaboration and commitment to organizing a scientific congress with prestigious researchers. I also acknowledge Professor João Soares Carrola for the kind gift in preparing the logo of the congress. My last words go to the Scientific Letters editorial staff for their fruitful collaboration in preparing the book of abstracts.

On 2 and 3 May 2024 we expect to be organizing the III TOXRUN INTERNATIONAL CONGRESS, perusing the objectives of having Toxicology and One Health join to achieve societal impact. All authors with relevant work and sharing the same spirit of this holistic One Health vision for Toxicology will be welcome.

I wish you a pleasant congress,

Cordial greetings

Ricardo Jorge Dinis-Oliveira

President of the Organizing Committee

Further Reading

1. Dinis-Oliveira, R.J. No Boundaries for Toxicology in Clinical Medicine: One Health, One Society and One Planet for All of Us. *J. Clin. Med.* **2023**, *12*, 2808.

SCIENTIFIC PROGRAMME

APRIL 27

Session I
A new era for Toxicology: All sciences interlinked

Chair: Filipa Grosso (UCIBIO-FFUP), Nuno Vieira e Brito (TOXRUN-IUCS), Vítor Seabra (TOXRUN-IUCS)

09h00 ***Opening Session*** | Ricardo Dinis-Oliveira, Cláudia Ribeiro, Diana Dias da Silva, Ana Raquel Freitas & Vítor Seabra, TOXRUN Management Board

09h30 ***Keynote session:*** The One Health concept under the perspective of a toxicologist. | Félix Carvalho, UCIBIO-Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Farmácia da Universidade do Porto

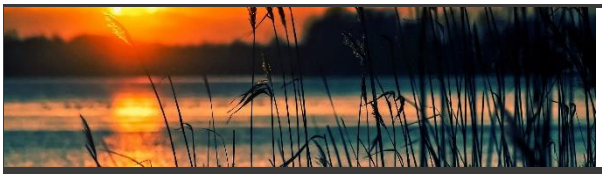
10h15 A century of research on environmental carcinogens: From legacy to emerging pollutants. | Pedro M. Costa, UCIBIO-Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Ciências e Tecnologia - Nova, Lisboa

10h45 ***Coffee break & Poster viewing***

11h15 Microbiome intra-host evolution in health and disease. | Isabel Gordo, Instituto Gulbenkian de Ciência, Lisboa

11h45 Animal production: challenges of being a black sheep. | Luís Pinho, ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Porto; SVA-Serviços Veterinários Associados, Porto

12h30 ***Lunch***



Session II
Mind and body: Triggers for the onset of disease

Chair: Inês Pádua (TOXRUN-IUCS), Helena Carmo (UCIBIO-FFUP), Hassan Bousbaa (UNIPRO-IUCS)

14h00 Cognitive enhancement in aging and neurodegeneration: a molecular mechanism turning an environmental pollutant into a critical brain mediator. | João Laranjinha, Faculdade de Farmácia da Universidade de Coimbra

14h30 Iodine intake and cognitive functioning in Portuguese school-age children. | Irene Carvalho, Faculdade de Medicina da Universidade do Porto

15h00 What is needed for allergic children? The one health approach. | André Moreira, Faculdade de Medicina da Universidade do Porto, Instituto de Saúde Pública da Universidade do Porto

15h45 ***Coffee break & Poster viewing***

16h15 ***Presentation of selected oral communications (Sessions I and II)***

OC 01 | Dynamics and effects of plastic contaminants' assimilation in gulls. Sara Veríssimo, MARE-Universidade de Coimbra

OC 02 | Dietary replacement of fishmeal with polychaete meal (Alitta virens) impacts European seabass acute stress response. Rafaela Costa, CIIMAR

OC 03 | Insights into the phylogeny, resistome, virulome and host adaptation from Gardnerella genome analysis. Pedro Teixeira, UCIBIO – Applied Molecular Biosciences Unit, Department of Biological Sciences, Faculty of Pharmacy, University of Porto)

OC 04 | Novel insights into healthy humans' faecal carriage of enterococci: Enterococcus lactis is as a dominant highly bacteriocinogenic species. Ana Cristina Santos, Faculdade de Farmácia da Universidade do Porto

OC 05 | The unknown acute toxicity of the antibiotic Sulfamethoxazole. Bárbara Diogo, ICBAS, CIIMAR, FCUP

OC 06 | The AHR: from a xenobiotic sensor to a Pattern Recognition Receptor, playing a role in immunity to infection and drug-therapy. Pedro Alves, CIIMAR

OC 07 | Cardiac mitochondrial dynamics, autophagy and regeneration are stirred by doxorubicin in old CD-1 mice. Sofia Brandão, Faculdade de Farmácia da Universidade do Porto

OC 08 | Nutrition and food safety & sustainability. Nuno Vieira e Brito, TOXRUN

18h00 ***Closing of the first day***

APRIL 28

Session III
Environmental risk assessment and climate changes: New challenges

Chair: Cristina Couto (TOXRUN-IUCS), João Carrola (CITAB-UTAD), José Carlos Andrade (TOXRUN-IUCS)

09h00 Environmental pollution and the success of antibiotic resistant bacteria. | Célia Manaia, Universidade Católica Portuguesa

09h30 Portugal at the crossroads of climate change: projections and threat to the sustainability of water resources. | João Andrade dos Santos, CITAB-Centro de Investigação e Tecnologias Agroambientais e Biológicas, Universidade de Trás-os-Montes e Alto Douro

10h00 Assessment of water treatments and conservation of aquatic ecosystems for environmental and human well-being. | Laura Guimarães, CIIMAR-Centro Interdisciplinar de Investigação Marinha e Ambiental

10h45 ***Coffee break & Poster viewing***

11h15 Microplastics in the environment - sources, fates and effects. | João Costa, CESAM-Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro

11h45 ***Presentation of selected oral communications***

OC 09 | Fighting occupational risks among Portuguese Wildland Firefighters: Looking at cytogenetic effects. Filipa Esteves, University of Porto

OC 10 | Hedgehogs (Erinaceus Europaeus) as bioindicatots of heavy metal(loid) pollution. Catarina J. Baptista, Universidade de Trás-os-Montes e Alto Douro

OC 11 | Seabirds as bioindicators of anthropogenic and chemical pollution. Diana Matos, Universidade de Coimbra

OC 12 | Bioinsecticide SPINTOR®: detrimental effects on earthworms Eisenia fetida at different levels of biological organization. Alexandre Moreira, ICBAS/CIIMAR

OC 13 | Occurrence of microplastics in water, feed, and tissues of European seabass produced in recirculating aquaculture system (RAS). Ricardo Matias, ICBAS/CIIMAR

12h45 ***Lunch***

Session IV
Psychoactive substances: New trends in misuse, monitoring and health impact

Chair: Vânia Vilas Boas (INL), Carolina Pereira (TOXRUN), Joaquim Monteiro (TOXRUN)

14h00 The impact of analytical techniques and methodologies in Forensic Toxicology. | Pedro Costa, Instituto Nacional de Medicina Legal e Ciências Forenses, Porto

14h30 Drug checking as a monitoring tool: an European perspective. | Daniel Martins, Kosmicare, TEDI - Trans European Drug Information

15h00 Impact of psychoactive substances in the neuroimmune crosstalk. | Teresa Summavielle, I3S - Instituto de Investigação e Inovação em Saúde

15h45 ***Coffee break & Poster viewing***

16h15 Special Invited Talk: Functional improvement in MS patients through changes in lipid profile after EGCG and coconut oil treatment. | Jose Orti, UCV - Universidad Católica de Valencia

16h35 ***Presentation of selected oral communications***

OC 14 | In vitro permeability of MDPV across Caco-2 cells for assessment of intestinal absorption and enantioselectivity. Ana Sofia Almeida, Faculdade de Farmácia da Universidade do Porto

OC 15 | The influence of glutathione pathway modulators on the cytotoxicity of sunitinib, doxorubicin and bortezomib in AC16 cardiac cells. Cláudia Oliveira, Faculdade de Farmácia da Universidade do Porto

OC 16 | Comparative Analysis of the Toxicity Profile of Eleven Consumer-Relevant Nanomaterials in Human Intestinal and Placental Barrier Cells. Joana Pires, Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto

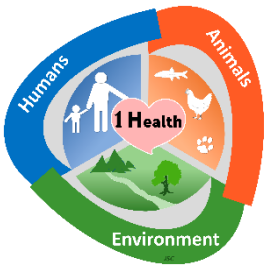
OC 17 | 4-Chloroethcathinone (4-CEC) in a single binge exposure triggers immediate and long sustained cognitive dysfunction in mice. Cristina de Mello Sampayo, iMed.ULisboa

OC 18 | Inhibitory activity of psilocybin/psilocin towards the enzymes of the cytochrome P450 (CYP450): An in vitro evaluation. Andreia Brito da Costa, TOXRUN

OC 19 | Drug poisoning profile in Portugal: a special focus on antipsychotics. André RTS Araújo, Research Unit for Inland Development (UDI), Polytechnic Institute of Guarda; TOXRUN; LAQV, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto

OC 20 | Acute toxicity screening of 4-chloroaniline in freshwater standard species. Daniela Fonseca Costa Rebelo, ICBAS/FCUP/CIIMAR

18h20 ***Awards Presentation and Closing Ceremony*** **|** Ricardo Dinis-Oliveira, Cláudia Ribeiro, Diana Dias da Silva, Ana Raquel Freitas & Vítor Seabra, TOXRUN Management Board



II TOXRUN INTERNATIONAL CONGRESS 2023

NO BOUNDARIES FOR TOXICOLOGY:
ONE HEALTH, ONE SOCIETY, ONE PLANET

27-28 APRIL, 2023 | PORTO, PORTUGAL

INVITED
SPEAKERS

INVITED SPEAKERS

SESSION I
A NEW ERA FOR TOXICOLOGY: ALL SCIENCES INTERLINKED

SPEAKERS

FÉLIX CARVALHO
UCIBIO-UNIDADE DE CIÊNCIAS BIOMOLECULARES APLICADAS, FACULDADE DE FARMÁCIA DA UNIVERSIDADE DO PORTO

PEDRO COSTA
UCIBIO-UNIDADE DE CIÊNCIAS BIOMOLECULARES APLICADAS, FACULDADE DE CIÊNCIAS E TECNOLOGIA - NOVA, LISBOA

ISABEL GORDO
INSTITUTO GULBENKIAN DE CIÊNCIA, LISBOA

LUÍS PINHO
ICBAS-INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR, PORTO; SVA-SERVIÇOS VETERINÁRIOS ASSOCIADOS, PORTO

SESSION II
MIND AND BODY: TRIGGERS FOR THE ONSET OF DISEASE

SPEAKERS

JOÃO LARANJINHA
FACULDADE DE FARMÁCIA
DA UNIVERSIDADE DE COIMBRA

IRENE CARVALHO
FACULDADE DE MEDICINA
DA UNIVERSIDADE DO PORTO

ANDRÉ MOREIRA
FACULDADE DE MEDICINA
DA UNIVERSIDADE DO PORTO,
INSTITUTO DE SAÚDE PÚBLICA
DA UNIVERSIDADE DO PORTO

SESSION III
ENVIRONMENTAL RISK ASSESSMENT AND CLIMATE CHANGES: NEW CHALLENGES

SPEAKERS

CÉLIA MANAIA
UNIVERSIDADE CATÓLICA PORTUGUESA

JOÃO ANDRADE DOS SANTOS
CITAB-CENTRO DE INVESTIGAÇÃO E TECNOLOGIAS AGROAMBIENTAIS E BIOLÓGICAS, UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

LAURA GUIMARÃES
CIIMAR-CENTRO INTERDISCIPLINAR DE INVESTIGAÇÃO MARINHA E AMBIENTAL

JOÃO COSTA
CESAM-CENTRO DE ESTUDOS DO AMBIENTE E DO MAR, UNIVERSIDADE DE AVEIRO

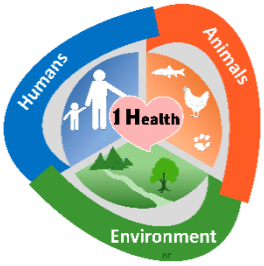
SESSION IV
PSYCHOACTIVE SUBSTANCES: NEW TRENDS IN MISUSE, MONITORING AND HEALTH IMPACT

SPEAKERS

PEDRO COSTA
INSTITUTO NACIONAL DE MEDICINA LEGAL E CIÊNCIAS FORENSES, PORTO

DANIEL MARTINS
KOSMICARE, TEDI - TRANS EUROPEAN DRUG INFORMATION

TERESA SUMMAVIELLE
I3S - INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE



II TOXRUN INTERNATIONAL CONGRESS 2023

NO BOUNDARIES FOR TOXICOLOGY:
ONE HEALTH, ONE SOCIETY, ONE PLANET

27-28 APRIL, 2023 | PORTO, PORTUGAL

SELECTED **ORAL COMMUNICATIONS**

Oral Communication 1

Dynamics and effects of plastic contaminants' assimilation in gulls

S. N. Veríssimo [1,*], S. C. Cunha [2], J. O. Fernandes [2], M. Casero [3], M. T. Vieira [4], J. A. Ramos [1], A. C. Norte [1] and V. H. Paiva [1]

[1] University of Coimbra, MARE − Marine and Environmental Sciences Centre, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[2] LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3] Wildlife Rehabilitation and Investigation Center (RIAS) – Associação ALDEIA, Ria Formosa Natural Park, Olhão, Portugal
[4] University of Coimbra, CEMMPRE, Department of Mechanical Engineering, R. Luís Reis Santos, 3030-788 Coimbra, Portugal

* Correspondence: snverissimo471@gmail.com

https://doi.org/10.48797/sl.2023.33

Abstract

Background: Opportunistic animals such as gulls are often associated with anthropogenic activities [1,2], and therefore highly susceptible to plastic ingestion and stomach chemical leaching [3]. Yet, such chemical process is still poorly studied and there is almost no information regarding potential hazardous effects in animal physiological processes. Even though it was already reported that gulls accumulate Polybrominated Diphenyl Ethers (PBDEs) [4], there is no information linking plastic ingestion with leaching and accumulation of these chemicals in different tissues. Moreover, it is still not fully known how PBDEs affect gulls' health and stress parameters. **Objective:** To evaluate: 1) how BDE99 leaches from ingested plastics into tissues, and 2) how this leaching will impact the individuals' immune status, nervous transmission and physiological stress parameters. **Methods:** We fed yellow-legged/black-backed gulls (*Larus michahellis*/*Larus fuscus*) in captivity with plastic pellets containing BDE99 (PBDE congener). BDE99 was measured in gulls' brain, preen oil, liver and fat tissues. Erythrocyte sedimentation rate, glutathione peroxidase activity, plasma antioxidant capacity and reactive oxygen metabolites and levels of cholinesterase and acetylcholinesterase activity were measured in blood samples. Cholinesterase activity levels were measured in the brain. **Results**: Gulls fed with plastics showed significantly higher concentrations of BDE99 in their fat and brain. There was a tendency for plastic-fed gulls to exhibit a more impaired health, yet only values of cholinesterase and acetylcholinesterase in plasma were significatively reduced at the end of the experiment. Cholinesterase in brain also tended to decrease in plastic-fed gulls. **Conclusions**: Our results indicate a clear relation of plastic ingestion with chemical leaching, a process occurring even if plastic stays in the stomach for a short period of time and that gulls' health can be affected, particularly gulls' neurofunction. Our results should have wider implications to understand the impacts of plastic contaminants' assimilation in vertebrates.

Keywords: *Larus* spp.; plastic ingestion; polybrominated diphenyl ethers (PBDEs); chemical leaching; toxicity

Acknowledgments

We acknowledge the support of the Foundation for Science and Technology, I.P. (FCT) for the fellowship granted to Sara N. Veríssimo, SFRH/BD/145827/2019.

References

1. Calado, J.G., Veríssimo, S.N., Paiva, V.H., Ramos, R., Vaz, P.T., Matos, D., Pereira, J., Lopes, C., Oliveira, N., Quaresma, A., et al.. Influence of fisheries on the spatio-temporal feeding ecology of gulls along the western Iberian coast. Mar. Ecol. Prog. Ser. (**2021**) *661*, 187–201. 10.3354/meps13601.
2. de Faria, J.P., Vaz, P.T., Lopes, C.S., Calado, J.G., Pereira, J.M., Veríssimo, S.N., Paiva, V.H., Gonçalves, A.M.M., and Ramos, J.A.. The importance of marine resources in the diet of urban gulls. Mar. Ecol. Prog. Ser. (**2021**) *660*, 189–201. 10.3354/meps13599.
3. Tanaka, K., Watanuki, Y., Takada, H., Ishizuka, M., Yamashita, R., Kazama, M., Hiki, N., Kashiwada, F., Mizukawa, K., Mizukawa, H., et al.. In Vivo Accumulation of Plastic-Derived Chemicals into Seabird Tissues. Curr. Biol. (**2020**) *30*, 723-728.e3. 10.1016/j.cub.2019.12.037.
4. Técher, R., Houde, M., and Verreault, J.. Associations between organohalogen concentrations and transcription of thyroid-related genes in a highly contaminated gull population. Sci. Total Environ. (**2016**) *545–546*, 289–298. 10.1016/j.scitotenv.2015.12.110.

Oral Communication 2

Dietary replacement of fishmeal with polychaete meal (*Alitta virens*) impacts European seabass acute stress response

R. S. Costa [1,3,*], M. Monteiro [1], L. Thoresen [2], K. Kousoulaki [2] and L. M. P. Valente [1,3]

[1] CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208, Matosinhos, Portugal
[2] Nofima, Nutrition and Feed Technology department, Kjerreidviken 16, 5141 Fyllingsdalen, Norway
[3] ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

* Correspondence: rafaelasilvacosta.rsc@gmail.com

https://doi.org/10.48797/sl.2023.49

Abstract

Background: Reducing the incorporation of fisheries-derived ingredients in aquafeeds is a major trend to support aquaculture's sustainable growth. Due to its high protein content and biochemical composition, polychaete meal (PM) is emerging as a viable alternative to fishmeal and potential functional ingredient [1]. **Objective:** This study aimed to evaluate the physiological response of *Dicentrarchus labrax* (European seabass) to acute stress, after feeding with diets containing increasing levels of PM (*Alitta virens*). **Methods:** A fishmeal-based diet (FM, control) and three diets containing PM at 2.5% (PM2.5), 5% (PM5), and 10% (PM10), to replace 10-40% of fishmeal, were fed to seabass juveniles for 93 days. Upon trial conclusion, fish underwent a stress challenge: 1-minute air exposure followed by 5-minute overcrowding. After 1 hour of recovery, plasma and liver samples were collected. Plasma metabolite levels, immune parameters and liver oxidative status of stressed fish were compared to those of non-stressed fish (n = 18). **Results:** Stress induced a significant increase in all biomarkers analyzed, regardless of the dietary treatment. Plasma glucose levels were significantly higher in fish fed PM10 compared to fish fed FM, independently of stress. Accordingly, cortisol levels were not affected by diet. Although basal plasma lactate levels were unaffected by diet, levels after stress were significantly higher in fish fed PM2.5 compared to fish fed FM. PM also impacted liver redox status. In relation to the control, basal GST activity was significantly higher in fish fed PM diets, as was glutathione content in fish fed PM2.5 and PM5. Nonetheless, differences among dietary groups were no longer observed after stress. **Conclusions:** Our results suggest dietary replacement of fishmeal with PM can modulate European seabass acute stress response. For further insight into the impact of PM on fish robustness, other oxidative stress biomarkers and innate immunity parameters will be analyzed and discussed.

Keywords: European seabass; polychaete meal; fish welfare; functional feeds; stress resistance

Acknowledgments

This research was funded by Project SIDESTREAM, subsidized through the ERA-NET BLUE BIOECONOMY COFUND by FCT – Foundation for Science and Technology (BLUEBIO/0005/2019). CIIMAR acknowledges FCT funding (UIDB/04423/2020).

References

1. Wan, A.H.L.; Snellgrove, D.L.; Davies, S.J. A comparison between marine and terrestrial invertebrate meals for mirror carp (*Cyprinus carpio*) diets: Impact on growth, haematology and health. *Aquaculture Research* **2017**, *48*, 5004-5016.

Oral Communication 3

Insights into the phylogeny, resistome, virulome and host adaptation from *Gardnerella* genome analysis

P. Teixeira [1,2,*], M. Sousa [1,2], F. Grosso [1,2], T. Ribeiro [1,2,3] and L. Peixe [1,2,3]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] CCP – Culture Collection of Porto, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

* Correspondence: up685449@up.pt

https://doi.org/10.48797/sl.2023.50

Abstract

Background: *Gardnerella* spp. is often seen as evidence of vaginal pathologies, although members of this genus can also be found in the urinary and vaginal microbiota of asymptomatic women [1,2], making its role in the urogenital tract unclear. **Objective:** To assess phylogenomic and functional analysis of *Gardnerella* genus. Resistance (ARGs) and virulence genes (Vg) was also explored to unveil their role in health and urogenital disease development. **Methods:** Twenty-nine *Gardnerella* isolates from urine (U, n=22) and vaginal swabs (VSn=7) of women [24 asymptomatic, 5 with overactive bladder (OAB)] were identified by cpn60 and WGS (NovaSeq 6000; Illumina). Together with118 genomes from public databases (from U (n=35), VS (n=82) blood (n=1), and unknown samples (n=2)), we used TYGS platform and fastANI for species identification. Phylogenomic and pangenome analyses were performed using anvi'o v7.1 and Roary. Vg were annotated with COG and KEGG databases, and ARGs with AMRFinder Plus. Vg presence/absence outoputs were analyzed with R. **Results:** 4 species and 10 genomic species were identified, 41% of the collection being comprised by *G. vaginalis*. ANI and dDDH values are insufficient for distinguishing *Gardnerella species*. Pangenome was composed by 4537 gene clusters and the core genome of by 514. Differences in carbohydrates and amino acid metabolism and absence of correlation of functions with human body sites or disease were observed. The virulome and *Gardnerella* species/genomospecies exhibit are intertwined. ARG to aminoglycosides (*aph(3')-Ia*), macrolides (*mefA*, *msrD*, *ermX*), tetracyclines (*tetM*, *tetL*), lincosamide and streptogramins (*lsaC*) were detected in several isolates. Open pangenomes were observed in *G. vaginalis*, *G. leopoldii*, *G. swidsinskii* and GG3. **Conclusions:** *Gardnerella* comprises 4 species and 10 genomic species. Core genome analysis, ANI and dDDH are recommended for appropriate species assignment. *Gardnerella* species/genomospecies are associated with a particular set of VFs and metabolic functions. ARGs with clinical relevance were also observed in different *Gardnerella* species.

Keywords: *Gardnerella*; pangenome; urogenital; microbiome

Acknowledgments

This work is financed by FCT (UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020). Márcia Sousa and Filipa Grosso are supported by FCT (2020.05038.BD, DL57/2016/CP1346/CT0034, respectively), Teresa Ribeiro by UCIBIO (UIDP/QUI/04378/2020), Pedro Teixeira by the project NORTE-01-0145-FEDER-000052, "Healthy&ValorFOOD – Towards healthy and valorized foods, assuring their safety, authenticity and sustainability".

References

1. Vaneechoutte, M.; Guschin, A. Emended description of *Gardnerella vaginalis* and description of *Gardnerella leopoldii* sp. Nov., *Gardnerella piotii* sp. nov. and *Gardnerella swidsinskii* sp. nov., with delineation of 13 genomic species within the genus *Gardnerella*. *IJSEM* **2019**, *69*, 679-687.
2. Castro, J.; Jefferson, K. Genetic Heterogeneity and Taxonomic Diversity among Gardnerella Species. *Trends Microbiol* 2020, *28*, 3, 202–211.

Oral Communication 4

Novel insights into healthy humans' faecal carriage of enterococci: *Enterococcus lactis* is as a dominant highly bacteriocinogenic species

A. C. Almeida-Santos [1,2,*], B. Duarte [1,2], M. J. Teixeira [3], A. P. Tedim [4,5], C. Novais [1,2], L. Peixe [1,2] and A. R. Freitas [1,2,3]

[1] UCIBIO/REQUIMTE, Applied Molecular Biosciences Unit, Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[2] Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Portugal
[3] TOXRUN, Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[4] Grupo de Investigación Biomédica en Sepsis – BioSepsis, Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain
[5] Centro de Investigación Biomédica en Red en Enfermedades Respiratorias (CIBERES, CB22/06/00035), Instituto de Salud Carlos III, Madrid, Spain

* Correspondence: acfasantos@gmail.com

https://doi.org/10.48797/sl.2023.51

Abstract

Background: *Enterococcus lactis* (Elts) [former *Enterococcus faecium* (Efm) clade-B] has been greatly associated with human colonization, but its epidemiology is unknown since this reclassification [1]. We aimed to assess the contemporary faecal carriage of enterococci species among healthy-humans (HH) in Portugal and get novel insights about Efm/Elts differences. **Methods:** Fifty-one faecal samples (29-women/22-men;18-85/~45-years) from HH in Northern Portugal (February-July 2022) were processed by enrichment/selection steps with/without ampicillin, vancomycin or linezolid. Efm, Elts and other species were identified by PCR [2, 3] and antibiotic-susceptibility by disk-diffusion/broth-microdilution (EUCAST/CLSI). Representatives/sample (n=40) were characterized by Whole-Genome-Sequencing/CGE-tools, including a homemade bacteriocins(bac) database. Qualitative bacteriocin production/sensitivity was performed in sequenced Efm/Elts and selected clinical VREfm (vancomycin-resistant-Efm) and Elts (all-against-all) using the soft-agar-overlay technique. **Results:** All samples carried *Enterococcus* (n=337), with most containing Elts-73% ($p<0.05$) and/or *E.faecalis* (Efs)-61% and variable occurrence for Efm-45%, *E.hirae*-16%, and/or other species (<2%). Samples (24% multidrug-resistant) included isolates resistant to erythromycin [73%;*erm(B)/msr(C)*], tetracycline [63%;*tet(M)/tet(L)*], high-level-streptomycin (22%;*ant(6)-Ia/str*), chloramphenicol (12%;*cat/fexA/fexB/optrA/poxtA*), quinupristin-dalfopristin (12%), high-level-gentamycin [4%;*aac(6')-Ie-aph(2'')-Ia*] and linezolid (4%-*optrA/poxtA*;MIC50/MIC90 4-mg/L). Acquired linezolid-resistance genes were detected in two samples: *optrA* (one *E. thailandicus*; MIC=8-mg/L) and *optrA+poxtA* (ST128-Efm; MIC=8-16-mg/L). Typical bacteriocins and plasmids from clinical Efm/Efs were scarce. Elts (2-5 bac; 100%-bac-genes+) and Efm (0-9; 71%-bac+) shared bacteriocins (e.g.,entP/entQ) contrasting with others exclusive of Efs (0-2;22%-bac+). No isolate could inhibit all or be inhibited by all, but the ones with more bacteriocins were less inhibited. Most Efm/Elts showed no activity against each other but ~30%/each inhibited most strains tested, including VREfm. **Conclusions:** Elts is one predominant enterococci gut species that would be misidentified as Efm without an accurate Efm/Elts distinction. Elts co-exist with Efm in the intestine, but specific Efm/Elts inhibiting most strains tested may contribute to microbiota restoration after antibiotic treatments. Linezolid-resistance genes finding is worrisome, suggesting an environmental/food-chain role in this acquisition since they were not described in enterococci from Portuguese hospitals before.

Keywords: *Enterococcus lactis*; linezolid resistance; bacteriocins; *optrA*; *poxtA*

Acknowledgments

This work received financial support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the exploratory project EXPL/SAU-INF/0261/2021.We acknowledge FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) for the national funds received through the projects UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences, project LA/P/0140/2020 of the Associate Laboratory, Institute for Health and Bioeconomy (i4HB). A.C.A.-S. gratefully acknowledges an UI/BD/151317/2021 fellowship granted by FCT/MCTES through national funds.

References

1. Belloso Daza, M.V., C. Cortimiglia, et al., Genome-based studies indicate that the Enterococcus faecium Clade B strains belong to Enterococcus lactis species and lack of the hospital infection associated markers. *Int J Syst Evol Microbiol,* **2021**. 71(8).
2. Belloso Daza, M.V., A.C. Almeida-Santos, et al., Distinction between Enterococcus faecium and Enterococcus lactis by a gluP PCR-Based Assay for Accurate Identification and Diagnostics. *Microbiol Spectr,* **2022.** 10(6): p. e0326822.
3. Jackson, C.R., P.J. Fedorka-Cray, and J.B. Barrett, Use of a genus- and species-specific multiplex PCR for identification of enterococci. *J Clin Microbiol*, **2004.** 42(8): p. 3558-65.

Oral Communication 5

The unknown acute toxicity of the antibiotic sulfamethoxazole

B. S. Diogo [1,2,3,*], S. Rodrigues [2,3] and S. C. Antunes [2,3]

[1] ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[2] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N, 4450-208 Matosinhos, Portugal
[3] FCUP - Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre S/N, 4169-007, Porto, Portugal

* Correspondence: barbara.diogo@edu.fc.up.pt

https://doi.org/10.48797/sl.2023.52

Abstract

Background: Sulfamethoxazole (SMX) is one of the most used antibiotics in the last 50 years, applied to humans, veterinary medicines, and aquaculture purposes [1]. Detected in different environmental matrices this antibiotic can pose a risk to aquatic ecosystems and contribute to the spread of antimicrobial resistance [2,3]. In addition, SMX has already been included in the 3rd Priority Substances Watch List to be monitored in inland surface waters throughout the European Union under the Water Framework Directive [2]. Regarding the available literature, different studies report the toxicity of SMX to several aquatic organisms, however, these data are quite discrepant and incomplete. **Objective:** Evaluate the acute toxicity effects of SMX in aquatic model organisms from different trophic levels. **Methods:** Standard acute ecotoxicological assays were performed [4–7], evaluating the inhibition of the bioluminescence of the bacterium *Aliivibrio fischeri*, growth inhibition of microalgae *Raphidocelis subcapitata* and macrophyte *Lemna minor*, and immobilization and reproductive effects in the microcrustacean *Daphnia magna*. Additionally, sub-individual parameters were also evaluated in *L. minor* and *D. magna*. **Results:** Overall, the different species showed different sensitivities to SMX, where preliminary results revealed that *R. subcapitata* was the most sensitive organism. SMX was harmful to *A. fischeri* [EC_{50} (30 min) = 79.67 mg/L], and toxic to *L. minor* [EC_{50} (7d) = 2.11 mg/L; causing also lipid peroxidation]. In addition, was harmful to *D. magna* [EC_{50} (48h) = 68.75 mg/L], causing significant alterations in sub-individual parameters (e.g., neurotoxicity), and reproductive endpoints, namely a decrease in the rate of population increase at 45 mg/L of SMX. **Conclusions:** These results highlight the effects that SMX can cause in non-target organisms. Considering that the detection of SMX is increasing considerably in various environmental matrices, it is essential to continue monitoring the behavior of this antibiotic in the environment and its long-term effects on ecosystems.

Keywords: sulfamethoxazole; ecotoxicity; acute exposure; multispecies

Acknowledgments

This work was supported by the strategic programs UIDB/04423/2020 and UIDP/04423/2020. Sara Rodrigues and Sara Antunes are hired through the Regulamento do Emprego Científico e Tecnológico—RJEC from the Portuguese Foundation for Science and Technology (FCT) program (2020.00464.CEECIND and CEECIND/01756/2017, respectively). Bárbara S. Diogo was supported by a FCT PhD grant (ref. 2022.10505.BD).

References

1. Ho, J.M.W.; Juurlink, D.N. Considerations When Prescribing Trimethoprim–Sulfamethoxazole. *Canadian Medical Association Journal* **2011**, *183*, 1851.
2. Cortes, L.; Marinov, D.,; Sanseverino, I.,; Cuenca, A.,; Niegowska, M.,; Rodriguez, E.; Lettieri, T., *Selection of Substances for the 3rd Watch List under the Water Framework Directive*; Luxembourg, **2020**.
3. Kovalakova, P.; Cizmas, L.; McDonald, T.; Marsalek, B. Occurrence and Toxicity of Antibiotics in the Aquatic Environment: A Review. *Chemosphere* **2020**, *251*.
4. OECD *Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test*; **2011**.
5. OECD *Test No. 221: Lemna Sp. Growth Inhibition Test*; **2006**.
6. OECD *Test No. 202: Daphnia Sp. Acute Immobilisation Test*; **2004**.
7. Microbics, M. *Microtox Manual - a Toxicity Handbook*; Microbics Corporation, **1992**; Vol. I–VI.

Oral Communication 6

The AHR: from a xenobiotic sensor to a Pattern Recognition Receptor, playing a role in immunity to infection and drug-therapy

P. Moura-Alves [1,2,*]

[1] IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
[2] i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

* Correspondence: pmouraalves@i3s.up.pt

https://doi.org/10.48797/sl.2023.53

Abstract

Background: The interaction between a bacterial pathogen and its host can be viewed as an "*arms race*" in which each participant continuously responds to the evolving strategies of the other partner. A mechanism allowing bacteria to rapidly adapt to such changing circumstances is provided by density-dependent cell-to-cell communication known as *Quorum Sensing* (QS). QS involves a hierarchy of signalling molecules, which in pathogenic bacteria is associated with biofilm formation and virulence regulation. **Objective:** We hypothesized that if a host sensor can detect and differentiate between bacterial QS molecules and their expression patterns, it will allow hosts to customize their immune responses according to the stage and state of infection. **Methods:** We have implied *in vitro* and *in vivo* assays (e.g., mouse, zebrafish) to evaluate how the host senses and responds to bacterial QS. **Results:** We found that the Aryl Hydrocarbon Receptor (AHR), a well-recognized receptor in the field of Toxicology, plays a role as an Innate Immune sensor. The AHR is able to sense diverse microbial-derived ligands and regulate different host defence mechanisms, according to the status and type of infection [1]. AHR modulation depends on the relative abundances of different QS molecules, whereby their quantitative assessment enables the host to sense bacterial community densities that may have distinct gene expression programs and infection dynamics. **Conclusions:** This study brought together concepts of immunology, which focused on the mobilization of defence mechanisms that combat invading pathogens, and concepts of toxicology focused on detoxification responses to inactivate toxins. We propose that by spying on bacterial *quorum*, the AHR acts as a major sensor of infection dynamics, capable of orchestrating host defence according to the *status quo* of the infection [1, 2]. Furthermore, our studies implicate the AHR in antibiotic resistance, whereby AHR activation by both drugs and infection impacts therapeutic efficacy [3].

Keywords: AHR; PRR; host-microbe interactions; quorum-sensing; antibiotic resistance

Acknowledgments

This research was funded by H2020-WIDESPREAD-2018-951921- ImmunoHUB, European Research Council under the Horizon 2020 program of the European Commission, grant 311371, Helmholtz BioInterfaces in Technology and Medicine (BIFTM) program of Karlsruhe Institute of Technology, International Max Planck Research School for Infectious Diseases and Immunology, and the Max Planck Society.

References

1. Moura-Alves, P., et al., *AhR sensing of bacterial pigments regulates antibacterial defence.* Nature, 2014. **512**(7515): p. 387-92.
2. Moura-Alves, P., et al., *Host monitoring of quorum sensing during Pseudomonas aeruginosa infection.* Science, 2019. **366**(6472).
3. Puyskens, A., et al., *Aryl Hydrocarbon Receptor Modulation by Tuberculosis Drugs Impairs Host Defense and Treatment Outcomes.* Cell Host Microbe, 2020. **27**(2): p. 238-248 e7.

Oral Communication 7

Cardiac mitochondrial dynamics, autophagy and regeneration are stirred by doxorubicin in old CD-1 mice

S. R. Brandão [1,2,3,*], A. Reis-Mendes [1,2], M. J. Neuparth [4,5], F. Carvalho [1,2], R. Ferreira [3] and V. M. Costa [1,2]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal
[2] UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal
[3] LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[4] Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sports, University of Porto, R. Dr. Plácido da Costa 91, 4200-450 Porto, Portugal; Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal
[5] TOXRUN - Toxicology Research Unit, University Institute of Health Sciences, CESPU, R. Central de Gandra, 4585-116 Gandra, Portugal

* Correspondence: sofiarbrandao@ua.pt

https://doi.org/10.48797/sl.2023.54

Abstract

Background: The chemotherapeutic agent doxorubicin (DOX) has been widely used in the treatment of solid tumors and hematological malignancies [1]. However, serious adverse side effects have emerged in patients treated with this drug, notably cardiotoxicity [2]. Moreover, aging is a risk factor for the development of cardiovascular diseases in cancer treated patients [3]. **Objective:** We herein aimed to evaluate the molecular effects of DOX on the cardiac muscle of old CD-1 mice. **Methods:** Old CD-1 male mice (19 months) were administered with a pharmacologically relevant cumulative dose of 9 mg/kg DOX (DOX group) or saline (CTRL group), distributed intraperitoneally for three weeks (biweekly). The experiments were performed with the approval of the national competent authorities (DGAV, reference nº 0421/000/000/2016). Animal welfare was monitored daily. Two months after the last drug or saline administration, mice were sacrificed for collection of blood and heart. **Results:** Serum glucose concentration was decreased after DOX administration, but no other differences were seen in serum markers evaluated. Regarding the heart, DOX increased the activity of citrate synthase (CS), suggesting increased mitochondrial density. Moreover, the content of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α) was decreased after DOX, pointing to decreased mitochondrial biogenesis. In parallel, the content of Beclin1 and microtubule-associated protein light chain 3 (LC3B) was decreased in DOX group, highlighting lower activation of autophagy. In addition, the content of mast/stem cell growth factor receptor Kit (SCFR) was increased after DOX, pointing to activation of cardiac regeneration. **Conclusions:** This work showed that even a low cumulative dose of DOX affects the cardiac muscle in multiple pathways requiring further studies to find new molecular mechanisms as to clinically address the cardiotoxicity induced by this anticancer agent.

Keywords: cardio-oncology; doxorubicin; mitochondrial biogenesis; autophagy; cardiac regeneration

Acknowledgments

This research was funded by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the projects UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. SRB and ARM acknowledge FCT, I.P. and European Social Fund for theirs grants (SFRH/BD/138202/2018, SFRH/BD/129359/2017). VMC acknowledges her grant (SFRH/BHD/110001/2015), received by Portuguese national funds through FCT, I.P., under the Norma Transitória DL57/2016/CP1334/CT0006.

References

1. Colombo, A. *et al*. Cardiac Complications of Chemotherapy: Role of Biomarkers. *Curr Treat Options Cardiovasc Med* **2014**, *16*, 313-8.
2. Brandão, S.R. *et al*. Insights on the molecular targets of cardiotoxicity induced by anticancer drugs: A systematic review based on proteomic findings. *Metabolism* **2022**, *134*, 155250.
3. Nowakowska, M. *et al*. Cardiovascular risk factors among cancer patients qualified for systemic treatment. *Arch Med Sci* **2020**, *16*, 1295-1303.

Oral Communication 8

Sustainability of the Portuguese autochthonous hens breeds: characterization of the productive system (eggs)

M. Meira [1], I. Afonso [1,2], V. Ribeiro [3], R. Dantas [3,4] and N. V. Brito [2,3,5,*]

[1] Agrarian Higher School, Polytechnic Institute of Viana do Castelo, 4990-706 Refóios do Lima, Portugal
[2] CISAS - Center for Research and Development in Agrifood Systems and Sustainability, Polytechnic Institute of Viana do Castelo, 4900-347 Viana do Castelo, Portugal
[3] AMIBA - Associação dos Criadores de Bovinos de Raça Barrosã, 4730-264 Vila Verde, Portugal
[4] University Institute of Health Sciences, CESPU, CRL., 4585-116 Gandra PRD, Portugal
[5] TOXRUN - Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra

* Correspondence: nuno.brito@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.55

Abstract

Background: The conservation of animal genetic resources represents an opportunity for the promotion of local genetic resources with benefits for marginal areas that have economic, cultural, social, and environmental potential, scientific use and that contribute to the sustainable preservation of biodiversity [1, 2]. In Portugal, four autochthonous chicken breeds have been recognized as being at risk of extinction, bred under traditional production systems, as dual-purpose animals for meat and eggs [2,3]. Consumer concern regarding the sustainability of production and animal welfare has strongly increased the demand for eggs and meat that are produced through alternative and extensive farming methods [4,5]. **Objective:** The aim of this study was to characterize the yield performance of indigenous Portuguese hens and evaluate the physicochemical composition of the eggs. **Methods:** Records were taken from hens bred in AMIBA farm with several flocks, sorted by breed. The production cycle was controlled during 2 years. The protein and mineral contents of the yolk and albumen in 240 eggs, 60 per breed, were estimated; protein content was determined according to Kjeldahl method (ISO 937:1978), while the mineral composition (P, K, Ca, Mg, Na, Fe, and Zn) was determined in freeze-dried samples [6]. **Results:** The four native Portuguese breeds perform well under extensive systems, with Pedrês Portuguesa appearing to be the most efficient laying breed. Productivity is significantly influenced by the hens' age and season, in tandem with the rearing system. The physicochemical composition and mineral content differ between breeds and egg constituents, with a higher protein content compared to a commercial genotype. K, Ca, Fe, and Zn contents were superior in native breeds when compared to the commercial genotype. **Conclusions:** Local breeds offer opportunities to adapt livestock to low-input environments and the characterization of the quality traits shows a strong contribute to future forms of sustainable poultry production.

Keywords: sustainability; productivity; physicochemical composition; autochthonous breeds

Acknowledgments

This research was funded by NORTE-01-0145-FEDER-000043 - TECH project – Technology, Environment, Creativity and Health – Activity 2.3 AVITECH, Polytechnic Institute of Viana do Castelo (IPVC).

References

1. Castellini, C.; Dal Bosco, A. Animal welfare and poultry meat in alternative production systems (and ethics of poultry meat production). In **Poultry Quality Evaluation**; Woodhead Publishing: Duxford, UK, 2017; pp. 335–357.
2. Brito, N.V.; Lopes, J.C.; Ribeiro, V.; Dantas, R.; Leite, J.V. Small scale egg production: The challenge of Portuguese autochthonous chicken breeds. *Agriculture* **2021**, *11*, 818.
3. Lordelo, M.; Cid, J.; Cordovil, C.M.D.S.; Alves, S.P.; Bessa, R.J.B.; Carolino, I. A comparison between the quality of eggs from indigenous chicken breeds and that from commercial layers. *Poult. Sci*. **2020**, *99*, 1768–1776.
4. Franzoni, A.; Gariglio, M.; Castillo, A.; Soglia, D.; Sartore, S.; Buccioni, A.; Mannelli, F.; Cassandro, M.; Cendron, F.; Castellini, C.; et al. Overview of native chicken breeds in Italy: Small scale production and marketing. *Animals* **2021**, *11*, 629.
5. Pettersson, I.C.; Weeks, C.A.; Wilson, L.R.M.; Nicol, C.J. Consumer perceptions of free-range laying hen welfare. *Br. Food J.* **2016**,*118*, 1999–2013.
6. A.P. Vale, J. Santos, N.V. Brito, V. Peixoto, R. Carvalho, E. Rosa, M.B.P.P. Oliveira, Light influence in the nutritional composition of Brassica oleracea sprouts. **Food Chemistry** *2015*, 178, 292–300.

Oral Communication 9

Fighting occupational risks among Portuguese wildland firefighters: looking at cytogenetic effects

F. Esteves [1,2,3,*], K. Slezakova [4], J. Madureira [1,2,3], J. Vaz [5,6], A. Fernandes [7], M. Carmo [4], S. Morais [8], J. P. Teixeira [1,2,3] and S. Costa [1,2,3]

[1] Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, nº 321, 4000-055 Porto, Portugal
[2] EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal
[3] Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal
[4] LEPABE-ALiCE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[5] CIMO, Instituto Politécnico de Bragança, Campus Santa Apolónia 5300-253 Bragança, Portugal
[6] SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[7] UICISA: E, Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[8] REQUIMTE/LAQV-Instituto Superior de Engenharia, Instituto Politécnico do Porto, Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

* Correspondence: filipa.esteves@insa.min-saude.pt

https://doi.org/10.48797/sl.2023.56

Abstract

Background: Evidence linking wildland firefighters' occupational exposure and health outcomes is still limited. Cytogenetic endpoints have long been applied in the surveillance of human genotoxic exposures and early effects of genotoxic carcinogens. Therefore, it is of utmost importance to clarify the exposure-induced cytogenetic effects concerning wildland firefighters' occupational exposure at different time points (Pre-fire season and fire season). **Objective:** Here, we aim to evaluate the cytogenetic levels in buccal cells among a group of wildland firefighters during a Pre-fire season, considering both the i) influence of self-reported variables (e.g., lifestyle) on buccal micronucleus cytome assay (BMCyt) outcomes and ii) the cytogenetic damage in exfoliated buccal cells considering the estimated inhalation doses to particulate matter (PM) in non-fire work settings. **Methods:** A total of 176 northern Portuguese wildland firefighters (82% males; mean age of 37.5 ± 10.9) were recruited during the pre-fire season of 2021. Relevant information was obtained through a self-administered questionnaire. Genomic instability was assessed for 172 northern Portuguese wildland firefighters by BMCyt. PM_{10} and $PM_{2.5}$ inhalation doses (indoor/outdoor) were estimated for a group of 80 firefighters based on methods described elsewhere [1]. **Results:** Some lifestyle variables (e.g., daily consumption of vegetables) shown to have a protective role on some BMCyt endpoints ($p<0.05$), whereas others such coffee consumption or being part of Permanent Intervention Teams (full-time firefighters) presented a negative impact ($p<0.05$). No significant association was found between estimated inhaled doses of PM_{10} and $PM_{2.5}$ (mean 1.73 ± 0.43 μg kg^{-1} and 0.53 ± 0.21 μg kg^{-1}, correspondingly) and BMCyt endpoints. **Conclusions:** The characterization of a population is a very important step to have a broad perspective of the potential risk factors that may influence the studied endpoints in further analysis. Surveillance based on (bio)monitoring programs may be a crucial tool to identify firefighters at high risk for developing adverse health outcomes.

Keywords: biomonitoring; occupational exposures; risk assessment

Acknowledgments

This work received financial support from the project PCIF/SSO/0017/2018 by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. Filipa Esteves, the recipient of the PhD grant UI/BD/150783/2020, is supported by FCT and by the European Social Fund (ESF). Joana Madureira, under the grant SFRH/BPD/115112/2016, is supported by FCT and by ESF, through Programa Operacional Capital Humano (POCH). This work is financed by national funds through the FCT within the scope of projects UIDB/04750/2020 and LA/P/0064/2020. Additional support was received from LA/P/0045/2020 (ALiCE) and UIDB/00511/2020 - UIDP/00511/2020 (LEPABE), UIDB/00690/2020 - UIDP/00690/2020 (CIMO) and SusTEC (LA/P/0007/2021).

References

1. Slezakova, K., et al., Indoor particulate pollution in fitness centres with emphasis on ultrafine particles. *Environ Pollut* **2018**, 233, 180-193.

Oral Communication 10

Hedgehogs (*Erinaceus europaeus*) as bioindicators of heavy metal(loid) pollution

C. Jota Baptista [1,2,3,4,*], F. Seixas [1,5], J. M. Gonzalo-Orden [3], C. Patinha [6], P. Pato [6], E. Ferreira da Silva [6], M. Casero [7], E. Brazio [8], R. Brandão [9], D. Costa [9], T. L. Mateus [5,10,11] and P. A. Oliveira [1,2]

[1] Departamento de Ciências Veterinárias, Escola de Ciências Agrárias e Veterinárias (ECAV), Universidade de Trás-os-Montes e Alto Douro, UTAD, Vila Real, Portugal
[2] Centro de Investigação das Tecnologias Agroambientais e Biológicas (CITAB), UTAD, Vila Real, Portugal
[3] Instituto de Biomedicina (IBIOMED), Universidad de León, León, Espanha
[4] Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[5] Centro de Ciência Animal e Veterinária (CECAV), AL4AnimalS, UTAD, Vila Real, Portugal
[6] GeoBioTec, Departamento de Geociências, Universidade de Aveiro, Aveiro, Portugal
[7] RIAS-ALDEIA - Wildlife Rehabilitation and Research Centre, Parque Natural da Ria Formosa, Olhão, Portugal
[8] Centro de Recuperação dos Animais Silvestres de Lisboa (LxCRAS), Parque Florestal de Monsanto, Lisboa, Portugal
[9] CERVAS-ALDEIA - Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens, Gouveia, Portugal
[10] CISAS-Centre for Research and Development in Agrifood Systems and Sustainability, Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal
[11] EpiUnit – Instituto de Saúde Pública da Universidade do Porto, Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal

* Correspondence: catabap@hotmail.com

https://doi.org/10.48797/sl.2023.57

Abstract

Background: Heavy metal(loid)s pollution is a One Health concern [1]. Hedgehogs (*Erinaceus europaeus*) are promising candidates for biomonitoring programs, due to their habits, abundance, distribution and resilience [2]. **Objective:** This work aims to evaluate heavy metal(loid)s pollution, using *E. europaeus* as a bioindicator. **Methods:** Necropsies of 46 hedgehogs from three distinct rescue centres (CERVAS, LxCRAS and RIAS) were performed. Provenance and clinical data (when available) were recorded. Sex and age group were estimated. Liver, kidney and external spines (2-10 grams) were collected and stored under -20ºC. Internal organs were completely freeze-dried for two days at -56ºC (LaboGene CoolSafe®) and stored frozen until further analysis. Spines were washed in an ultrasound machine (Sonorex RK 106®) and dried overnight in an oven (55ºC). Acid digestion was performed in a digestion plate (DigiPrep-MS®) and metal(loid)s concentrations (As, Cd, Cr, Cu, and Pb) were determined with ICP-MS. Liver and kidney were also collected for histopathology routine examination. **Results:** High levels of Cu were found in the kidney (24.74±21.05 mg kg^{-1} dry weight [dw]) and liver (35.66 ± 19.65 mg kg^{-1}dw), with some animals passing 100 mg kg^{-1} dw, which is a high value for insectivores [3]. Significant correlations have been found between spines and liver and between spines and kidney, for Co ($p<0.001$, in both organs) and Pb ($p=0.020$ and $p=0.019$), suggesting spines as a non-invasive sample to access internal metal(loid) concentrations. Biliary hyperplasia was the most frequent lesion observed (36%)- Animals presenting biliary hyperplasia show higher levels of metal(loid)s, with a significant difference for Cd ($p=0.007$) and Co ($p=0.019$). **Conclusions:** Further research, including different locations and organs, is mandatory to comprehend the real impact of metal(loid)s pollution in different Portuguese locations, under a One Health perspective.

Keywords: trace elements; hedgehog; environmental contamination; One Health

Acknowledgments

This work was funded by FCT (Fundação para a Ciência e Tecnologia) under the PhD scholarship 2021.04520.BD. Teresa Letra Mateus was supported by UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by FCT.

References

1. Ding, C.; Chen, J.; Zhu, F.; Chai, L.; Lin, Z.; Zhang, K.; Shi, Y. Biological Toxicity of Heavy Metal(Loid)s in Natural Environments: From Microbes to Humans. *Front. Environ. Sci*. **2022**; *10:*920957.
2. Jota Baptista, C.; Seixas, F.; Gonzalo-Orden, J.M.; Oliveira, P.A. Can the European hedgehog (*Erinaceus europaeus*) be a sentinel for One Health concerns? *Biologics*. **2021**; *1(1),* 61-69.
3. D'Havé, H.; Scheirs, J.; Mubiana, V.K.; Verhagen, R.; Blust, R.; de Coen, W. Non-Destructive Pollution Exposure Assessment in the European Hedgehog (Erinaceus Europaeus): II. Hair and Spines as Indicators of Endogenous Metal and As Concentrations, *Env. Pollution* **2006**; *142(3):*438-48.

Oral Communication 11

Seabirds as bioindicators of anthropogenic and chemical pollution

D. M. Matos [1,*], J. A. Ramos [1], L. C. Brandão [2], F. Bessa [1], V. Silva [1], I. Rodrigues [3], S. Antunes [3], I. dos Santos [1], J. Coentro [1], S. C. Cunha [4], J. O. Fernandes [4], L. A. E. Batista de Carvalho [2], M. P. M. Marques [2,5], S. Santos [1] and V. H. Paiva [1]

[1] University of Coimbra, MARE – Marine and Environmental Sciences Centre / ARNET – Aquatic Research Network, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[2] University of Coimbra, Molecular Physical-Chemistry R&D Unit, Department of Chemistry, 3004-535 Coimbra, Portugal
[3] Biosfera Cabo Verde, Sul do Cemitério, Rua 5 – Caixa Postal 233, São Vicente, Cabo Verde
[4] LAQV/REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[5] University of Coimbra, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

* Correspondence: dianammatos92@outlook.com

https://doi.org/10.48797/sl.2023.58

Abstract

Background: Marine pollution, caused by anthropogenic debris, is a significant environmental issue that has detrimental effects on marine ecosystems [1]. Finding suitable sentinel species of the human impacts on the oceans, is imperative. As top predators, seabirds are considered sentinels of the marine environment [2]. **Objective:** To provide quantitative data about the distinctive prevalence of anthropogenic pollution on seabirds, five species with different trophic and foraging ecology inhabiting the tropical Atlantic region were used. **Methods:** The occurrence of anthropogenic debris was assessed using faeces as a proxy for ingestion. Particles were chemically analysed using micro-Fourier transform infrared spectroscopy (mFTIR) [3]. Moreover, preen oil and plasma samples were analysed for Polybrominated Diphenyl ethers (PBDEs) and methoxylated PBDEs (MeO-PBDEs), through with gas chromatography–mass spectrometry (GC-MS/MS) [4]. **Results:** There were found 438 items suspected of anthropogenic origin, mostly fragments and fibres in all species. *Phaethon aethereus* (PA n=61), *Calonectris edwardsii* (CE n=119) and *Sula leucogaster* (SL n=82) presented the highest frequency occurrence (FO: 51%, 49%, 48%), while *Bulweria bulwerii* (BB =86) and *Puffinus lherminieri boydi* (PB n=86) the lowest (FO: 30%, 36%) of anthropogenic particles. Particles revealed a high diversity of polymers, from cellulosic particles to synthetic plastics. PBDEs and MeO-PBDEs were detected in all species and matrices, whereby preen oil had higher concentrations and variety of congeners than plasma. PB had the lowest chemical concentrations in both tissues compared to other species. Moreover, it was not found any correlation between chemical compounds concentration and the number or occurrence of anthropogenic particles. However, particles and chemical compounds were more prevalent in species that explore areas associated to anthropogenic activities. **Conclusions:** Overall, anthropogenic pollution is transversal to all species, ranging from particle ingestion to chemical compounds. We considered that it is necessary to continue monitoring the impacts of global anthropogenic pollution considering the declines in seabirds' population.

Keywords: tropical seabirds; anthropogenic debris; PBDEs; foraging ecology; trophic ecology

Acknowledgments

This work received financial and logistic support for fieldwork campaigns, from the project Alcyon – Conservation of seabirds from Cabo Verde, coordinated by BirdLife International and funded by the MAVA foundation (MAVA17022; http://en.mava-foundation.org), through its strategic plan for West Africa (2017-2022). DMM was supported by Fundação para a Ciência e Tecnologia (FCT; SFRH/BD/143659/2019). Molecular Physical-Chemistry R&D Unit financed by FCT (UIDB/00070/2020; UIDP/00070/2020).

References

1. Lewis SL, Maslin MA. Defining the Anthropocene. Nature. **2015,** 519, 171–180.
2. Burger J, Gochfeld M. Marine Birds as Sentinels of Environmental Pollution. Ecohealth. **2004,**1, 263–274.
3. Bessa F, Kogel T, Frias J, Lusher A. Harmonized protocol for monitoring microplastics in biota. Micropoll-Multilevel assessment of microplastics and associated pollutants in the Baltic Sea View project. **2019**, 1–30.
4. Cruz R, Marques A, Casal S, Cunha SC. Fast and environmental-friendly methods for the determination of polybrominated diphenyl ethers and their metabolites in fish tissues and feed. Science of the Total Environment. **2019**, 646, 1503–1515.

Oral Communication 12

Bioinsecticide SPINTOR®: detrimental effects on earthworms *Eisenia fetida* at different levels of biological organization

A. Moreira [1,2,*], V. Nogueira [2,3], S. Bouguerra [3,4] and S. Rodrigues [2,3]

[1] ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[2] CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões | Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[3] FCUP, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto | Rua do Campo Alegre S/N. 4169-007 Porto, Portugal
[4] GreenUPorto, Sustainable Agrifood Production Research Center & Inov4Agro | Rua da Agrária 747, 4485-646 Vairão, Portugal

* Correspondence: up201805452@up.pt

https://doi.org/10.48797/sl.2023.59

Abstract

Background: The increasing interest in sustainable agricultural practices led to the use of biopesticides instead of synthetic ones [1]. SPINTOR® (SPI) is a commercialized bioinsecticide that employs spinosad as its active ingredient, a natural by-product obtained from the *Saccharopolyspora spinosa* fermentation [2]. Although effective in controlling several pests, SPI also can present inadvertent effects on non-target organisms in different environmental compartments [3]. *Eisenia fetida* is an example of soil fauna that can be affected and is frequently used as model organisms in soil ecotoxicology research. **Objective:** The objective of this study is to evaluate the potential ecotoxicity of SPINTOR® on several endpoints of *E. fetida*. **Methods:** The effect of SPI on the reproduction [4] and avoidance behavior [5] of *E. fetida* was assessed following standard protocols. A short-term exposure of 48 h was also carried out. The experimental design consisted in eight treatments of a natural soil spiked with different concentrations of SPI (0.00 up to 1.49 mg of active ingredient/kg of $soil_{dw}$). The concentrations were determined based on the application dose and environmental relevance. The *E. fetida* adults from the short-term exposure (48 h) and the reproduction assay (28 d) were further processed to assess several biochemical parameters, including biomarkers related to oxidative stress, energy reserves, neurotransmission, and genotoxic effects through the comet assay. **Results:** Initial findings indicate that chronic exposure of *E. fetida* causes alterations to its energy-related metabolic pathways and antioxidant defenses. And although no effects were observed in the number of juveniles produced at the end of the assay, *E. fetida* show a tendency to avoid the contaminated soil in the highest concentrations, as well as significant DNA damage was observed after 48 h of exposure. **Conclusions:** It can be inferred that SPINTOR® has a negative impact on the health and wellness of *E. fetida*, potentially impairing their crucial roles in terrestrial ecosystems.

Keywords: Oligochaeta; reproductive activity; genotoxicity; metabolic and physiological disorders

Acknowledgments

This research was funded by Foundation for Science and Technology, and by the Strategic Program UIDB/04423/2020 and UIDP/04423/2020. Sara Rodrigues is hired through the Regulamento do Emprego Científico e Tecnológico – RJEC from the FCT program (2020.00464.CEECIND). Verónica Nogueira thanks FCT for funding through program DL 57/2016 – Norma transitória. Sirine Bouguerra thanks the NORTE 2020 Program funded by FEDER through the project S4Hort Soil & Food (REF: Norte-01-0145-FEDER-000074) for the financial support of her investigation contract. This research was also funded by the Master's in Toxicology and Environmental Contamination, from Instituto de Ciências Biomédicas Abel Salazar (ICBAS).

References

1. Hole, D. G.; Perkins, A. J.; Wilson, J. D.; Alexander, I. H.; Grice, P. V.; & Evans, A. D.; Does organic farming benefit biodiversity? Biological Conservation **2005**, 122(1), 113-130.
2. MERTZ, F. P.; & Yao, R. C. Saccharopolyspora spinosa sp. nov. isolated from soil collected in a sugar mill rum still. International Journal of Systematic and Evolutionary Microbiology **1990** 40(1), 34-39.
3. Biondi, A., Mommaerts V.; Smagghe, G.; Viñuela, E.; Zappalà, L.; Desneux, N.; The non-target impact of spinosyns on beneficial arthropods. Pest Manag Sci. **2012**, 68(12):1523-36.
4. ISO. (2012). International Organization for Standardization Guideline 11268-2: Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction of *Eisenia fetida*/*Eisenia andrei*.
5. ISO. (2008). International Organization for Standardization Guideline 17512-1: soil quality—avoidance test for determining the quality of soils and effects of chemicals on behaviour—part 1: test with earthworms (*Eisenia fetida* and *Eisenia andrei*).

Oral Communication 13

Occurrence of microplastics in water, feed, and tissues of European seabass produced in recirculating aquaculture system

R. S. Matias [1,2,*], S. Gomes [1,2], L. G. A. Barboza [1,2], L. Guilhermino [1,2] and L. M. P. Valente [1,2]

[1] CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n. 4450-208 Matosinhos, Portugal
[2] ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

* Correspondence: ricardomatias.bio@gmail.com

https://doi.org/10.48797/sl.2023.60

Abstract

Background: Microplastics (MPs) in seafood are a global major concern to human health [1]. Due to MPs' ubiquitous presence in aquatic ecosystems, aquacultures are also susceptible to contamination from external or endogenous sources. Previous studies have reported MP occurrence in farmed organisms [2], but little is known about recirculating aquaculture systems (RAS), which provide an opportunity for sustainable seafood production under environmentally controlled conditions. **Objective:** The present study aimed to investigate MP occurrence in several body sites of European seabass (*Dicentrarchus labrax*) produced in a RAS, due to the high presence of plastic components. MP occurrence in two direct exposure routes, water and feed, was also evaluated. **Methods:** Water, feed, and 55 fish were collected from a RAS facility. MP retention in seabass was investigated in the gastrointestinal tract (GIT), gills, liver, and dorsal muscle [3]. Bioconcentration (BCF) and bioaccumulation (BAF) factors of MPs were estimated [4]. **Results:** MP concentrations in water and feed were 37.2±1.9 MP/L and 3.9±1.3 MP/g, respectively. In total, 422 MPs were recovered from seabass body sites: GIT presented the highest concentration (1.0±0.8 MP/g) and muscle the lowest (0.4±0.3 MP/g). All fish had MPs recovered from at least two of the analysed body sites. Black, blue, and transparent fibres made of regenerated cellulose and polyethylene terephthalate were the most common particles. Polymers linked to RAS components (e.g., polyethylene, polypropylene) occurred in low quantity, suggesting a limited system's contribution to overall MP levels. BCF and BAF values equal to 19.9 and 0.2, respectively, indicating MP bioconcentration in fish (i.e., >1), but no bioaccumulation. **Conclusions:** RAS-farmed seabass are susceptible to MPs through water and feed, potentially retaining MPs in tissues. MP occurrence in muscle highlights RAS-farmed fish as a potential dietary exposure pathway to humans, but further dedicated risk evaluation studies are required to fully ascertain the relevance of such finding for consumers.

Keywords: bioconcentration; microplastics; 'One Health' concept; recirculating aquaculture systems (RAS); seafood contamination

Acknowledgments

This research was supported by the project "ATLANTIDA – Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources" (NORTE-01-0145-FEDER-000040), funded by NORTE2020 through the European Regional Development Fund (ERDF). Also supported by FCT through the grant awarded to R. Matias (2022.10421.BD) and L.G.A. Barboza (CEEIND/2020/02573).

References

1. Garrido Gamarro, E.; Constanzo, V. Microplastics in food commodities – A food safety review on human exposure through dietary sources. *Food Saf. Qual. Ser.* **2022**, FAO, Rome, Italy, 1-124.
2. Zhou, A.; Zhang, Y.; Xie, S.; Chen, Y.; Li, X.; Wang, J.; Zou, J. Microplastics and their potential effects on the aquaculture systems: a critical review. *Rev. Aquac.* **2021,** 13(1): 719-733.
3. Guilhermino, L.; Martins, A.; Lopes, C.; Raimundo, J.; Vieira, L.R.; Barboza, L.G.A.; Costa, J.; Antunes, C.; Caetano, M.; Vale, C.; Microplastics in fishes from an estuary (Minho River) ending into the NE Atlantic Ocean. *Mar. Pollut. Bull.* **2021**, 173, 113008.
4. Miller, M.E.; Motti, C.A.; Hamann, M.; Kroon, F.J.; Assessment of microplastic bioconcentration, bioaccumulation and biomagnification in a simple coral reef food web. *Sci. Total Environ.* **2023**, 858, 159615.

Oral Communication 14

In vitro permeability of MDPV across Caco-2 cells for assessment of intestinal absorption and enantioselectivity

A. S. Almeida [1,2,3,4], B. Silva [3,4], F. Remião [3,4] and C. Fernandes [1,2,*]

[1] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal
[2] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, s/n, 4450-208 Matosinhos, Portugal
[3] UCIBIO—Applied Molecular Biosciences Unit, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal
[4] Associate Laboratory i4HB—Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal

* Correspondence: cfernandes@ff.up.pt

https://doi.org/10.48797/sl.2023.61

Abstract

Background: Synthetic cathinones, such as 3,4-methylenedioxypyrovalerone (MDPV), are widely studied chiral psychoactive substances [1]. Enantioselectivity studies on the toxicokinetic of synthetic cathinones are still scarce [2], namely on their ability to cross the intestinal membrane, which was evaluated only for methylone and pentedrone [3]. To isolate enantiomers for these studies, liquid chromatography (LC) using chiral columns has been the technique of choice [4]. Objective: The aims of this work were the semi-preparative enantioresolution of the enantiomers of MDPV and development and validation of an UHPLC-UV method to further evaluate the potential enantioselectivity in intestinal permeability using the Caco-2 cell line. **Methods:** A semi-preparative LC method using a polysaccharide-based column was optimized to obtain the enantiomers of MDPV. Caco-2 monolayers were exposed to each enantiomer and samples were collected and quantified by UHPLC-UV. For the validation of the UHPLC-UV method, specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) and samples' stability were evaluated [5]. **Results:** The enantiomers were successfully separated by the optimized LC method with good resolution (R_s of 1.7) and enantioselectivity (α of 1.4), being collected with high enantiomeric ratios (>95%) and recovery rates (>92%). Regarding the UHPLC-UV, high selectivity and good linearity were obtained ($r^2 > 0.999$). Acceptable accuracy (between 102-109%) and inter-day and intra-day precisions (CV < 15%) low LOD and LOQ values (0.063 µM and 0.19 µM, respectively) were also observed. Samples were stable for 6 weeks of storage in different temperatures (room temperature, 4 °C, -20 °C and -80 °C). **Conclusions:** The enantiomers of MDPV were found to be highly permeable across the Caco-2 monolayer and enantioselectivity was found for the P_{app} values in the basolateral (BL) to apical (AP) direction.

Keywords: Caco-2 cells; enantioselectivity; liquid chromatography; MDPV; permeability

Acknowledgments

This research was supported by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry—CIIMAR), and under the projects UIDB/MULTI/04378/2020, LA/P/0140/2020, PTDC/CTA-AMB/6686/2020. Ana Sofia Almeida acknowledges the BYT-Plus 2021/22 scholarship from CIIMAR and Amadeu Dias Foundation.

References

1. Almeida AS, Silva B, Silva JP, Pereira JA, Remião F, Fernandes C. Semi-Preparative Separation, Absolute Configuration, Stereochemical Stability and Effects on Human Neuronal Cells of MDPV Enantiomers. *Molecules.* **2023**;28(5):2121.
2. Silva B, Fernandes C, Guedes de Pinho P, Remião F. Chiral Resolution and Enantioselectivity of Synthetic Cathinones: A Brief Review. *J Anal Toxicol.* **2018**;42(1):17-24.
3. Silva B, Silva R, Fernandes C, Guedes de Pinho P, Remião F. Enantioselectivity on the absorption of methylone and pentedrone using Caco-2 cell line: Development and validation of an UHPLC method for cathinones quantification. *Toxicol Appl Pharmacol.* **2020**;395:114970.
4. Almeida AS, Silva B, Pinho PG, Remião F, Fernandes C. Synthetic Cathinones: Recent Developments, Enantioselectivity Studies and Enantioseparation Methods. *Molecules.* **2022**;27(7).
5. Almeida AS, Silva B, Remião F, Fernandes C. Assessment of the Permeability of 3,4-Methylenedioxypyrovalerone (MDPV) across the Caco-2 Monolayer for Estimation of Intestinal Absorption and Enantioselectivity. *Int J Mol Sci.* **2023**;24(3):2680.

Oral Communication 15

The influence of glutathione pathway modulators on the cytotoxicity of sunitinib, doxorubicin and bortezomib in AC16 cardiac cells

C. Vitorino-Oliveira [1,2,*], S. Kahremany [3], L. Nisim [3], M. Duarte-Araújo [4,5], F. Carvalho [1,2], A. Gruzman [3] and V. M. Costa [1,2]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] Department of Chemistry, Faculty of Exact Sciences, Bar-Ilan University, Ramat Gan Israel
[4] LAQV/REQUIMTE, University of Porto, Porto, Portugal
[5] Department of Immuno-Physiology and Pharmacology, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

* Correspondence: up202104039@up.pt

https://doi.org/10.48797/sl.2023.62

Abstract

Background: Cancer survivorship has grown over the years due to more efficacious treatments. Nonetheless, anticancer treatments present serious adverse effects, namely cardiotoxicity. Doxorubicin (DOX), a topoisomerase II inhibitor; sunitinib (SUN), a multikinase inhibitor; and bortezomib (BTZ), an inhibitor of proteasome, have their clinical use limited because of their cardiotoxicity. Cardiotoxicity is often linked to oxidative stress. Nuclear factor erythroid 2–related factor 2 (Nrf2) controls the expression of the antioxidant response element and is an important emerging regulator of oxidative stress response. Nrf2 regulates glutathione (GSH) homeostasis, being this the most important cellular antioxidant. **Objective:** To study the influence of Nrf2 activators, like SK-119 and SH-29, and GSH modulators, namely its precursor *N*-acetylcysteine (NAC) and the γ-glutamylcysteine synthetase inhibitor, L-buthionine sulfoximine (BSO) on the cytotoxicity of SUN, DOX and BTZ in human differentiated AC16 cardiac cells. **Methods:** AC16 cells were differentiated with horse serum and then exposed to different concentrations of SUN (1–20µM) or DOX (0.1–10µM) or BTZ (0.01–20µM) for 24 or 48h to assert their cytotoxicity. The two cytotoxicity assays done were the MTT reduction and neutral red uptake assays. Then, two concentrations of SUN (1 and 10µM), DOX (0.1 and 1µM) and BTZ (1 and 0.01µM) were co-incubated with BSO, NAC, SK-119 or SH-29. **Results:** A time and concentration-dependent cytotoxicity was observed in the anticancer therapies, being the degree of toxicity as follows: BTZ>DOX>SUN when using equimolar concentrations. Regarding data with Nrf2/GSH modulators, a small but significant protection was achieved with NAC towards the cytotoxicity of DOX. BSO had no effects on the cytotoxicity of the anticancer agents. SK-119 and SH-29 are still being evaluated, showing no relevant cytotoxicity in the micromolar range. **Conclusions:** This work showed that DOX, SUN and BTZ cause significant cytotoxicity in AC16 cells, being GSH pathways involved in DOX toxicity. Nonetheless, more studies are needed, being key to determine the role of Nrf2 on the cytotoxicity of these anticancer therapies.

Keywords: cardio-oncology; doxorubicin; sunitinib; bortezomib; glutathione; Nrf2

Acknowledgments

This research was funded by national funds from Fundação para a Ciência e a Tecnologia (FCT), in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences (UCIBIO) and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB. V.M.C acknowledges FCT for her grant (SFRH/BPD/110001/2015) that was funded under the Norma Transitória – DL57/2016/CP1334/CT0006.

References

1. Reis-Mendes, A., et al., Role of Inflammation and Redox Status on Doxorubicin-Induced Cardiotoxicity in Infant and Adult CD-1 Male Mice. Biomolecules, 2021. 11(11).

Oral Communication 16

Comparative analysis of the toxicity profile of eleven consumer-relevant nanomaterials in human intestinal and placental barrier cells

J. Pires [1,2,3,*], L. Moreira [1,2,3], J. P. Teixeira [1,2,3] and S. Fraga [1,2,3]

[1] Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), Rua de Alexandre Herculano, nº 321, 4000-053, Porto, Portugal
[2] EPIUnit – Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, nº 135, 4050-600, Porto, Portugal
[3] Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, nº 135, 4050-600, Porto, Portugal

* Correspondence: joanamapires.97@gmail.com

https://doi.org/10.48797/sl.2023.47

Abstract

Background: The growing number of items incorporating nanomaterials (NM) has prompted considerable concerns about human health and safety [1]. Metal nanoparticles, inorganic non-metallic, and carbon-based NM are among the types with the highest market volume [2]. **Objective:** The purpose of this study was to determine the effect of chemical composition [Ag, Au, TiO_2, SiO_2, and graphene oxide (nano_GO)], primary size (10, 30 and 60 nm AgNP and AuNP), crystal structure (TiO_2NP rutile/anatase and anatase), and surface coating (citrate and PEGylated AuNP) on potential toxicity to human intestinal (Caco-2) and placental (BeWo b30) epithelial cells. **Methods:** Changes in cell morphology, metabolic activity, plasma membrane integrity, intracellular ROS and ATP levels, and DNA integrity were assessed to investigate their potential toxicity at 24 h after exposure. **Results:** In both barrier models, the toxicity profile was similar, however placental were more sensitive than intestinal epithelial cells. Overall, NM may be ranked for cytotoxicity as AgNP > nano_GO > AuNP ~ TiO_2NP ~ SiO_2NP, with the effects becoming more evident at greater concentrations. The influence of size was more pronounced for AgNP than for AuNP, with the smaller nanoparticles producing higher cytotoxic effects. The cytotoxicity of AuNP was prevented by PEG capping. AgNP and nano_GO exposure markedly raised the levels of ROS, indicating that oxidative stress may play a role in their cytotoxicity. Except for 10 nm AuNP, every NM tested markedly increased intracellular ATP levels. One interesting finding was that a higher cytotoxic potential did not necessarily equate to a higher genotoxic potential, since only AgNP (classified as positive) and anatase TiO_2NP (classified as equivocal) caused DNA damage. **Conclusions:** Our findings alert to the potential risks associated with human barriers exposure to NM, where the physicochemical properties are important determinants of their toxicity. Additional research is needed for a deeper understanding of NM impact on human barriers.

Keywords: nanomaterials; *in vitro* toxicity; Caco-2 cells; BeWo b30 cells

Acknowledgments

This research was funded by the project NanoBioBarriers, grant number PTDC/MED-TOX/31162/2017, co-financed by the Operational Program for Competitiveness and Internationalization (POCI) through European Regional Development Funds (FEDER/FNR) and the Foundation for Science and Technology (FCT/MCTES). This work is financed by national funds through FCT – Foundation for Science and Technology within the scope of projects UIDB/04750/2020 and LA/P/0064/2020.

References

1. Najahi-Missaoui, W.; Arnold, R.D.; Cummings, B.S. Safe Nanoparticles: Are We There Yet?. *Int J Mol Sci*. **2020**, *22*.
2. Contado, C. Nanomaterials in consumer products: a challenging analytical problem. *Front. Chem.* **2015**, *3*.

Oral Communication 17

4-Chloroethcathinone (4-CEC) in a single binge exposure triggers immediate and long sustained cognitive dysfunction in mice

C. De Mello-Sampayo [1,2,*], M. Leitão [1], C. Gamito [1], D. Brites [1,2] and A. T. Lopes [3]

[1] Laboratory of Neuroinflammation, Signaling and Neuroregeneration, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal
[2] Faculty of Pharmacy, Universidade de Lisboa, Portugal
[3] FarmID, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

* Correspondence: csampayo@ff.ulisboa.pt

https://doi.org/10.48797/sl.2023.23

Abstract

Background: Synthetic cathinones (SC) are β-keto analogues of amphetamine. They usually produce short-lived stimulant effects, which trigger higher desire of re-dosing with potential risk of overdosing. Its consumption occurs frequently in the form of multiple administration during a single exposure-event (binge), mostly by young party attendees. Adolescence is a critical time of brain development, and any disruption has a high risk of inducing brain dysfunction. 4-chloroethcathinone (4-CEC), a briefly studied SC, has been identified in seized samples and linked to overdose cases [1,2], but its repercussions on cognitive functions have not been assessed, yet. Currently, there is limited information, even contradictory, on the short- and the long-term cognitive implications associated to SC use during adolescence, and even fewer when binge exposure is thought. Hence, this study aimed to demonstrate the short- and long-term effects of a single binge exposure to 4-CEC on cognitive and emotional functioning of young compared to adults. **Methods:** Young (1-month) and adult (6-months) mice were exposed to a single-binge (2x16mg/kg or 2x32mg/kg, ip, 2h interval) of 4-CEC or saline and the effects on learning/memory were assessed, by the Morris watermaze, at 24h, one and 6-months (long-term) post exposure. Effects on emotional behaviors were assessed at the same three time points, too. **Results:** The obtained results demonstrate that a single binge exposure to 4-CEC elicited, long lasting, learning and memory impairment, associated to anxiolytic behaviour and increased apathy. Most importantly, young mice seem to be more susceptible to 4-CEC than adult mice. **Conclusions:** These results alert the cognitive impact and on mental health that a 4-CEC single binge exposure may have, especially during adolescence, when brain plasticity is still occurring.

Keywords: synthetic cathinones; 4-chloroethcathinone; binge

Acknowledgments

This work was supported by the National funds from Fundação para a Ciência e Tecnologia (FCT): PTDC/SAU-TOX/32515/2017 to Á. T. Lopes, and in part by UIDB/04138/2020 and UIDP/04138/2020 to iMed.ULisboa.

References

1. Pieprzyca, E.; Skowronek, R.; Czekaj, P. Toxicological analysis of intoxications with synthetic cathinones. *J Anal Tox* **2022**, 46, 705-711.
2. Gatch, M.B.; Shetty, R.A.; Sumien, N.; Forster, M.J. Behavioral effects of four novel synthetic cathinone analogs in rodents. *Addict Biol* **2021**, 26, e12987.

Oral Communication 18

Inhibitory activity of psilocybin/psilocin towards the enzymes of the cytochrome P450 (CYP450): an *in vitro* evaluation

A. M. Brito-da-Costa [1,2,3,*], M. Silva-Carvalho [1,2,3], R. J. Dinis-Oliveira [1,2,3,4], Á. Madureira-Carvalho [1,5] and D. Dias da Silva [1,2,3]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences (IUCS) – CESPU, Gandra, Portugal
[2] UCIBIO/REQUIMTE, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] Associate Laboratory i4HB – Institute of Health and Bioeconomy, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[5] LAQV/REQUIMTE, Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Porto, Porto, Portugal

* Correspondence: a26127@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.63

Abstract

Background: Psilocybin is a hallucinogen produced by several "magic mushrooms" [1,2]. This prodrug is rapidly metabolized in the organism by alkaline phosphatases and esterases into psilocin, the active drug [1,2]. A scientific gap exists regarding the possible interactions between psilocybin/psilocin and CYP450 enzymes. Since the binding of drugs to CYP450 enzymes can interfere with the metabolism of other substrates leading to drug-drug interactions, this research topic is of utmost importance. **Objective:** This study aimed to assess potential inhibitory interactions between psilocybin/psilocin and CYP3A4, 2D6, 2B6 and 2A6. **Methods:** The *in vitro* assessment of CYP450 inhibition was performed using the Vivid®CYP450 screening kits, following the user's guide. Concentrations of psilocybin and psilocin ranged between 1.14×10^{-13} - 4 mM and 6.1×10^{-5} - 1 mM for CYP3A4; 1.71×10^{-13} - 8 mM and 6.1×10^{-5} - 1 mM for CYP2D6; 2.4×10^{-4} - 8 mM and 2.4×10^{-5} - 1 mM for CYP2B6; and 3.8×10^{-6} - 2 mM and 7.6×10^{-8} - 1 mM for CYP2A6, respectively. Each test condition was mixed with baculosomes expressing the specific CYP, Vivid® regeneration system, $NADP^+$, and a non-fluorescent substrate. Solvent and positive controls of inhibition, i.e., ketoconazole (CYP3A4), quinidine (CYP2D6), miconazole (CYP2B6) and tranylcypromine (CYP2A6,) were included. Fluorescence was measured for 60 minutes (Ex=415/20nm; Em=460/20nm) and the half-maximal inhibitory concentration (IC_{50}) calculated using GraphPad prism 9.3.0. For CYP3A4 and 2D6 a minimum of three independent experiments were performed, and two independent experiments for CYP2A6 and 2B6. **Results:** For psilocybin, IC_{50} values of 49.43 μM (CYP3A4), >1000 μM (CYP2D6 and 2B6), and >300 μM (CYP2A6) were attained. For psilocin, the following IC_{50} values were obtained: 2.12 μM (CYP3A4), 11.89 μM (CYP2D6), 0.99 μM (CYP2A6) and 4.05 μM (CYP2B6). **Conclusions:** The results suggest a potential for psilocin to be an inhibitor of all the enzymes evaluated, especially CYP2A6, contrary to psilocybin which seems to only have the potential to inhibit CYP3A4.

Keywords: pharmacokinetics; hallucinogens; magic mushrooms

Acknowledgments

This research was funded by i) FCT — Fundação para a Ciência e a Tecnologia in the scope of the PhD grant 2021.04999.BD, the projects UIDP/04378/2020 and UIDB/04378/2020 of the UCIBIO, and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB; ii) IINFACTS-CESPU in the scope of the project PsiloPharma_PI2RL_IINFACTS_2021; and iii) EUROX Pharma.

References

1. Brito-da-Costa, A. M.; Dias da Silva, D.; Madureira-Carvalho, Á.; Dinis-Oliveira, R. J., Psilocybin and magic mushrooms: Patterns of abuse and consequences of recreational misuse. In *Handbook of Substance Misuse and Addictions*, Patel, V. B.; Preedy, V. R., Eds. Springer Nature: Switzerland, 2022.
2. Dinis-Oliveira, R. J., Metabolism of psilocybin and psilocin: clinical and forensic toxicological relevance. *Drug Metab Rev* **2017**, 49 (1), 84-91.

Oral Communication 19

Drug poisoning profile in Portugal: a special focus on antipsychotics

A. R. T. S. Araújo [1,2,3,*], E. Gallardo [4,5] and F. Rato [6]

[1] Research Unit for Inland Development (UDI), Polytechnic Institute of Guarda, Guarda, Portugal
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[3] LAQV, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4] CICS-UBI, Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal
[5] Laboratory of Pharmaco-Toxicology, Ubimedical, University of Beira Interior, Covilhã, Portugal
[6] Centro de Informação Antivenenos (CIAV), Instituto Nacional de Emergência Médica, Lisboa, Portugal

* Correspondence: andrearaujo@ipg.pt

https://doi.org/10.48797/sl.2023.64

Abstract

Background: Acute poisoning is a major public and preventable global health problem, contributing to morbidity and mortality in many parts of the world. Most patients with acute poisoning are treated as outpatients in hospital emergency departments. Acute poisoning occurs soon after exposure to either single or multiple toxic substances. Poisoning cases may be intentional or unintentional. **Objective:** The aim of the present study was to carry out a drug poisoning profile in Portugal with a special focus on the antipsychotic drugs. **Methods:** This work describes the retrospective and descriptive analysis of the information reported to Centro de Informação Antivenenos (CIAV) from 2019 to 2021 [1]. Data analysis focused on the main toxic involved in poisonings. **Results:** In Portugal there is a prevalence of non-intentional poisonings, affecting more adults than children, the overwhelming majority occurring by ingestion. The main agents involved were medicines (60.4%), domestic/industrial products (21.7), biocides/phytopharmaceuticals (5.6%), substances of abuse (5.5%) and cosmetics (2.7%). Regarding drug poisonings, it was verified that the pharmacotherapeutic groups most involved were anxiolytics and sedative-hypnotics (27.4%), antipsychotics (11.1%), antidepressants (10.5%), non-steroidal anti-inflammatory drugs (8.1%) and paracetamol (7.8%). There was an increasing trend of poisonings with Central Nervous System (CNS) drugs in these 3 years, from 44.2% and 64.6%. The antipsychotics occupied the second position, with emphasis for quetiapine. This drug has become widely used for a variety of indications because of its favorable side effect and safety profile. Patients with an acute overdose of quetiapine may demonstrate central nervous system depression, sinus tachycardia, prolonged QTc interval, hypotension, coma, and seizures [2]. **Conclusions:** Since drug intoxications represent a public health problem, it is of utmost importance to highlight to the rational use of the medicines, namely CNS drugs, and therefore the need of the improvement in the prevention and education in this field is mandatory.

Keywords: poisoning; drugs; central nervous system drugs; antipsychotics

Acknowledgments

This research received no external funding.

References

1. Serviço Nacional de Saúde, CIAV – Instituto Nacional de Emergência Médica, https://www.inem.pt/category/servicos/centro-de-informacao-antivenenos/
2. Chen, J.A.; Unverferth, K.M.; Cheung, E.H. Delayed-Onset Seizure in a Mild Quetiapine Overdose: Report of a Case and Review of the Literature. *Case Rep. Psychiatry.* **2018**, *2018*, 1-3.

Oral Communication 20

Acute toxicity screening of 4-chloroaniline in freshwater standard species

D. Rebelo [1,2,3,*], S. C. Antunes [2,3] and S. Rodrigues [2,3]

[1] Abel Salazar Biomedical Sciences Institute (ICBAS) – University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[2] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N, 4550-208 Matosinhos, Portugal
[3] Department of Biology, Faculty of Sciences – University of Porto (FCUP), Rua do Campo Alegre S/N, 4169-007 Porto, Portugal

* Correspondence: up202210683@edu.icbas.up.pt

https://doi.org/10.48797/sl.2023.65

Abstract

Background: Aromatic amines are commonly used in the production of pigments, dyes, pharmaceuticals, pesticides, and laboratory chemicals [1]. Due to inappropriate discard and the ineffectiveness of wastewater treatment plants in removing these compounds, they are frequently detected in aquatic ecosystems [2], leading to 4-chloroaniline being considered a candidate for the 4th Watch List under the Water Framework Directive [3], to determine the risk it poses to the aquatic environment. **Objective:** To evaluate the biological effects (individual and sub-individual responses) of standard species from different trophic levels, after exposure to 4-chloroaniline. **Methods:** A toxicity screening was performed regarding the ecotoxicological effects on *Allivibrio fischeri* (bioluminescence inhibition), *Raphidocelis subcapitata* (growth inhibition), *Lemna minor* (growth inhibition and biomarker assessment) and *Daphnia magna* (immobilization/mortality, reproductive effects and biomarker assessment). **Results:** The 4-chloroaniline exposure showed an *A. fischeri* effect concentration of EC_{50}(30 min) = 1.99 mg/L. Preliminary results demonstrate that *R. subcapitata* should be the most sensitive organism. *L. minor* presented an EC_{50}(7 d) = 82.84 mg/L. *L. minor* sub-individual results showed a significant decrease (≥ 93.75 mg/L) in the photosynthetic pigments content; a significant increase in catalase and glutathione *S*-transferases activities; and a significant decrease in lipid peroxidation. *D. magna* showed an EC_{50}(48 h) = 0.102 mg/L and, after a subchronic exposure (10 days), a significant decrease in N1 fecundity was recorded above 13.89 µg/L. Catalase and Glutathione *S*-transferases activities were only significantly increased at 13.89 mg/L. **Conclusions:** Results showed that 4-chloroaniline has an ecotoxicologically relevant effect in aquatic organisms, with an impact on several biochemical pathways, ultimately affecting individuals (survival, growth and reproduction responses). To better understand the effects of exposure to 4-chloroaniline in non-target organisms, more studies (e.g., chronic assays) should be conducted to produce data to help policy decisions related to monitoring and regulating these compounds in surface waters.

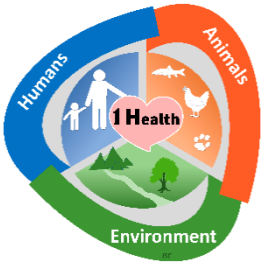
Keywords: ecotoxicology; aromatic amines; aquatic organisms; individual and sub-individual responses; biomarkers

Acknowledgments

This research was funded by Foundation for Science and Technology, and by the Strategic Program UIDB/04423/2020 and UIDP/04423/2020. Sara Antunes and Sara Rodrigues are hired through the Regulamento do Emprego Científico e Tecnológico – RJEC from the FCT program (CEECIND/01756/2017 and 2020.00464.CEECIND, respectively). Daniela Rebelo is supported by FCT PhD grants (2022.13777.B).

References

1. Boehncke, A.; Kielhorn, J.; Konnecker, G.; Pohlenz-Michel, C.; Mangelsdorf, I. Concise International Chemical Assessment Document 48 - 4-Chloroaniline; Publisher: World Health Organization Geneva, Switzerland, **2003**; pp. 1-62.
2. Zhou, L.J.; Rong, Z.Y.; Gu, W.; Fan, D.L.; Liu, J.N.; Shi, L.L.; Xu, Y.H.; Liu, Z.Y. Integrated Fate Assessment of Aromatic Amines in Aerobic Sewage Treatment Plants. *Environ Monit Assess* **2020**, *192*, doi:10.1007/S10661-020-8111-Y.
3. Cortes, L.G.; Marinov, D.; Sanseverino, I.; Cuenca, A.N.; Niegowska, M.; Rodriguez, E.P.; Stefanelli, F.; Lettieri, T. Selection of Substances for the 4th Watch List under the Water Framework Directive; Luxembourg, **2022**, doi:10.2760/01939.



II TOXRUN INTERNATIONAL CONGRESS 2023

NO BOUNDARIES FOR TOXICOLOGY:
ONE HEALTH, ONE SOCIETY, ONE PLANET

27-28 APRIL, 2023 | PORTO, PORTUGAL

POSTERS

Poster 1

Species richness of *Myxobolus* (Cnidaria, Myxozoa) parasites infecting thicklip grey mullet *Chelon labrosus* in the Douro River, Portugal

J. Guimarães [1], G. Casal [1,2], A. Alves [1], C. Araújo [3,4] and S. Rocha [1,4,*]

[1] School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Rua Jorge Viterbo Ferreira no. 228, 4050-313 Porto, Portugal
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[3] Faculty of Sciences (FCUP), University of Porto, Rua do Campo Alegre, s/n, FC4, 4169-007 Porto, Portugal
[4] Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Rua Alfredo Allen no. 208, 4200-135 Porto, Portugal

* Correspondence: srrocha@icbas.up.pt

https://doi.org/10.48797/sl.2023.66

Abstract

Background: Myxozoans are widespread cnidarian parasites that mostly infect fish and annelids as temporary and definitive hosts, respectively [1]. Mullets are a diverse fish group, which ubiquitous nature leaves vulnerable to parasitic infections [2]. **Objective:** This study aimed to acknowledge the myxozoan diversity infecting thicklip grey mullet *Chelon labrosus* in the Douro River estuary in northern Portugal. **Methods:** The internal and external organs of 13 specimens were macro- and microscopically examined. Cysts and infected tissues were individually photographed and processed for sequencing of the small subunit ribosomal gene (18S rDNA). Phylogenetic reconstructions were performed using maximum likelihood and Bayesian inference methodologies. **Results:** Eleven potentially new *Myxobolus spp*. were morphologically described and molecularly characterized. Additionally, a novel host, geographic region and morphometric profile was reported for *Myxobolus pupkoi* Gupta et al., 2022. Molecular comparisons further matched two of the novel *Myxobolus* sequences with sphaeractinomyxon types previously reported from marine oligochaetes in another Portuguese estuary. Phylogenetic analyses revealed the novel sequences clustering according to host affinity, with tree topologies resolving well-supported lineages of myxobolids infecting mullets from the genera *Chelon*, *Mugil*, *Crenimugil* and *Planiliza*. **Conclusions:** The elevated number of potentially novel *Myxobolus spp.* found in *C. labrosus* confirmed the successful hyperdiversification of these myxozoans in mullets [2], further reinforcing molecular-based comparisons as imperative for taxonomic descriptions. Morphometrical divergence between geographical isolates of *M. pupkoi* was hypothesized to correlate with adaptation to distinct abiotic factors and annelid communities. The formation of more than one *Chelon*-infecting lineage revealed that myxobolids entered this genus multiple times during their evolution. Lastly, the matching of two novel *Myxobolus* sequences with sphaeractinomyxon types reinforces the latter as specific life-cycle counterparts of mugiliform-infecting *Myxobolus* [3]. The large number of unmatched sphaeractinomyxon sequences positioned within the *Chelon* lineages suggests that *Myxobolus* diversity in Portuguese estuaries remains underestimated.

Keywords: 18S rDNA; diversity; life cycle

Acknowledgments

This research was funded by national funds through Foundation for Science and Technology (FCT), within the scope of the project PTDC_BIA-BMA_6363_2020, and the FCT employment contract 2022.06670.CEECIND.

References

1. Okamura, B.; Gruhl, A.; Bartholomew, J.L. An introduction to myxozoan evolution, ecology and development. In Myxozoan Evolution, Ecology and Development; Okamura, B., Gruhl, A., Bartholomew, J.L., Eds.; Springer International Publishing: Switzerland, 2015, pp. 1-20.
2. Rocha, S.; Casal, G.; Alves, Â.; Antunes, C.; Rodrigues, P.; Azevedo, C. Myxozoan biodiversity in mullets (Teleostei, Mugilidae) unravels hyperdiversification of *Myxobolus* (Cnidaria, Myxosporea). *Parasitol. Res.* **2019**, 118, 3279-3305.
3. Rocha, S.; Rangel, L. F.; Casal, G.; Azevedo, C.; Rodrigues, P.; Santos, M. J. Involvement of sphaeractinomyxon in the life cycle of mugiliform-infecting *Myxobolus* (Cnidaria, Myxosporea) reveals high functionality of actinospore morphotype in promoting transmission. *Parasitology* **2020**, 147, 1320-1329.

Poster 2

Molecular characterization of thyroid tumors of dogs – a multicentric Portuguese series

A. Monteiro [1,2,3], T. B. Gaspar [1,2,4], M. Pinto [1,2], I. Pires [3,5,6], P. Soares [1,2,7] and C. Tavares [1,2,8,*]

[1] Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, 4200-135 Porto, Portugal
[2] Instituto de Patologia e Imunologia Molecular da Universidade do Porto (Ipatimup), 4200-135 Porto, Portugal
[3] University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
[4] Departamento de Ciências Veterinárias (DCV), Escola Universitária Vasco da Gama (EUVG), 3020-210 Coimbra, Portugal
[5] CECAV-Veterinary and Animal Research Center, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal
[6] Department of Veterinary Science of the University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
[7] Medical Faculty of the University of Porto (FMUP), 4200-319 Porto, Portugal
[8] TOXRUN – Unidade de Investigação em Toxicologia, Instituto Universitário de Ciências da Saúde, CESPU, CRL, 4585-116 Gandra, Portugal

* Correspondence: catarina.tavares@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.67

Abstract

Background: The incidence of thyroid carcinoma (TC) in human population has been increasing worldwide, and it was estimated an incidence of 2.1% of cancer new cases in 2019 [1]. A smaller incidence is reported for canine population (1.1%) in 1995-2005 [2]. Diagnosis, prognosis, and management of human TC rely on mutation screening of *BRAF*, *RAS* genes, and *TERT* promoter. *BRAF*, *NRAS*, *HRAS*, and *KRAS* encode proteins that are key effectors of MAPK signaling pathway, an important kinase pathway, conserved in mammals [3]. **Objective: O**ur goal was to explore the canine TC's oncobiology, and to verify whether natural occurring canine TC could (or not) be set as suitable model to study its human homolog. **Methods:** We collected 57 samples (5 adenomas (9%), and 52 carcinomas (91%)), from which we performed DNA extraction from formalin-fixed paraffin-embedded tissues, PCR, and Sanger sequencing of exon 16 of BRAF (n = 49), exon 2 of NRAS (n = 41), exon 3 of HRAS (n = 41), and exon 3 (n = 31) and 4 (n = 20) of KRAS. **Results:** We detected silent mutations on *HRAS* (p.N47=) (n = 14/41, 34%) and *NRAS* (p.E63=) (n = 1/41, 2.4%), however, no mutations were found in the other genes. **Conclusions:** Our results corroborate those described by Campos et al. (2014) [4]. Nevertheless, both studies only evaluated the homologous regions of the hotspots of human most common TC mutations. We cannot exclude the hypothesis that in dogs, those genes can present activating mutations in other exons, different from human's hotspots.

Keywords: thyroid; tumours; dogs

Acknowledgments

The authors would like to thank Veterinary Oncology Network (Vet-OncoNet) for providing all the cases included in our study population. Additionally, we would like to thank Rúben Leite for the support provided during DNA extraction of FFPE tissues.

References

1. www.seer.cancer.gov
2. Katja L Wucherer, Vicki Wilke. Thyroid cancer in dogs: an update based on 638 cases. J Am Anim Hosp Assoc. 2010. 46, 249-54.
3. Cuenda, Ana. Mitogen-Activated Protein Kinases (MAPK) in Cancer. Reference Module in Biomedical Sciences (2018). 10.1016/B978-0-12-801238-3.64980-2.
4. M. Campos, M.M.J. Kool, S. Daminet, R. Ducatelle, G. Rutteman, H.S. Kooistra, S. Galac. Upregulation of the PI3K/Akt Pathway in the Tumorigenesis of Canine Thyroid Carcinoma. J.A. Mol. 2014, 28, 1814-1823.

Poster 3

Diagnostic methods of leishmaniosis in dogs: state-of-the-art

C. P. Penelas Costa [1,*], J. C. Prata [2,3], L. Cardoso [1,4] and T. G. Barroso [2,5]

[1] Animal and Veterinary Research Centre (CECAV), Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal
[2] TOXRUN—Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[3] Food Microbiology and Technology Laboratory, Department of Aquatic Production, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[4] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 5000-801 Vila Real, Portugal
[5] INESC TEC—Institute for Systems and Computer Engineering, Technology and Science, Campus da FEUP, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

* Correspondence: a33113@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.68

Abstract

Background: Canine leishmaniosis (CanL) is caused by *Leishmania infantum* transmitted by the bite of phlebotomine sand flies [1]. **Objective:** This systematic review aims at providing the most updated information about the laboratory diagnosis of leishmaniosis in dogs using traditional methods and innovative techniques. **Methods:** A bibliographic search was performed on 23 February 2023 in Scopus targeting all currently available literature up to 22 February 2023 using the following keywords: ("Canine leishmaniosis" OR "Leishmania" OR "Leishmaniosis in Dogs" OR "Visceral leishmaniasis" OR "cutaneous leishmaniasis" OR "leishmaniasis") AND ("traditional diagnosis" OR "biochemical profile" OR "clinical analysis" OR "complete blood count (CBC)" OR "cytology" OR "hemogram" OR "PCR" OR "serology" OR "urinalysis") AND ("innovative diagnostic techniques" OR "molecular diagnostic test" OR "rapid diagnostic tests" OR "spectroscopy POC") AND ("diagnostic performance"). **Results:** CanL laboratory diagnosis is traditionally carried out directly or indirectly, with methods including molecular techniques, quantitative serological methods, specific serology and also the evaluation of hematological and biochemical parameters [2]. Innovative techniques address point-of-care applications, e.g. DNA-probes based on gold nanoparticles. **Conclusions:** Traditional diagnostic methods vary in their simplicity of use, however, these methods require large sample volumes, which cannot always be collected from patients and may pose welfare concerns. Innovative techniques should provide high method accuracy while using low sample volumes and being reagentless, reducing costs and applying green chemistry principles.

Keywords: diagnosis; dogs; leishmaniosis; methods

Acknowledgments

This research received no external funding.

References

1. Torrent, E.; Planellas, M.; Ordeix, L.; Pastor, J.; Rodon, J.; Solano-Gallego L. Serum Symmetric Dimethylarginine as an Early Marker of Excretory Dysfunction in Canine Leishmaniosis (L. infantum) Induced Nephropathy. *Vet Med Int* **2018**, 7517359.
2. Salant, H.; Nachum-Biala, Y.; Feinmesser, B.; Perelmutter, M.; Baneth, G. Early onset of clinical leishmaniosis in a litter of pups with evidence of in utero transmission. *Parasit. Vectors* **2021**,*14*, 326.

Poster 4

Description of sphaeractinomyxon types (Cnidaria: Myxosporea) from marine oligochaetes in the Minho River estuary and nearby coastal area

C. Araújo [1,2,*], L. F. Rangel [2,3], G. Casal [4,5], D. Costa Araújo [3,6] and S. Rocha [1,4]

[1] Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Rua Alfredo Allen no. 208, 4200-135 Porto, Portugal
[2] Faculty of Sciences, University of Porto (FCUP), Rua do Campo Alegre, s/n, FC4, 4169-007 Porto, Portugal
[3] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos Porto, Portugal
[4] School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Rua Jorge Viterbo Ferreira no. 228, 4050-313 Porto, Portugal
[5] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[6] Aquamuseu do Rio Minho, Parque do Castelinho, 4920-290 Vila Nova de Cerveira, Portugal

* Correspondence: catarinacoutada2@gmail.com

https://doi.org/10.48797/sl.2023.69

Abstract

Background: Myxosporeans are important fish endoparasites with a complex life cycle that involves the production of actinospores in annelid hosts. More than 2,400 myxosporean species are presently known, yet only about 60 have their complete life cycle described [1]. Difficulties in the annelid sampling and examination, namely from the marine environment, and typically low prevalence of infection, hinder our knowledge of myxosporean-annelid interactions [2]. **Objective:** This study focused on the detection of myxosporean parasites present in the marine annelid communities of the Minho River lower estuary and nearby coastal area, seeking to unravel their biodiversity and potentially allow the clarification of new life cycles. **Methods:** Estuarine sediment and coastal substrates were collected monthly from "Sapal do Coura" in the Minho River lower estuary and at Moledo beach, respectively. Annelids collected from these sampling sites were microscopically examined for the detection of actinospore development. Actinospores were morphologically characterized and prepared for DNA extraction and sequencing of the small subunit ribosomal gene (18S rDNA). For annelid identification, the 16S rRNA gene of mitochondrial DNA (mtDNA) was amplified and sequenced. Sequence assembly and maximum likelihood phylogenetic analysis were performed using MEGA X. **Results:** Six morphologically and molecularly distinct types of sphaeractinomyxon were found infecting the coelomic cavity of marine oligochaetes belonging to the family Naididae. BLASTn revealed three of these types as novel records, one of which could be molecularly inferred as the life cycle counterpart of the mugiliform-infecting myxosporean *Myxobolus labrosus*. Maximum likelihood retrieved all novel sequences positioned within the monophyletic clade of mugiliform-infecting *Myxobolus*. **Conclusions:** This study expands the known diversity of sphaeractinomyxon types, reinforcing naidids as preferred hosts for these myxosporeans in marine environments. The strengthening of the correlation between sphaeractinomyxon and mugiliform-infecting *Myxobolus* reinforces the functionality of this actinospore morphotype in promoting transmission to mullet hosts [3].

Keywords: Annelida; Naididae; life cycle; mugiliform-infecting *Myxobolus*; 18S rDNA

Acknowledgments

This research was funded by national funds through Foundation for Science and Technology (FCT) within the scope of the MSc fellowship grant attributed to C. Araújo (Fellow_BIM/FCT_Proj2021/i3S/26120601/2022) through the project PTDC/BIA-BMA/6363/2020, and the FCT employment contracts 2022.06670.CEECIND and CEECIND/03501/2017.

References

1. Eszterbauer, E., Atkinson, S., Diamant, A., Morris, D., El-Matbouli, M., Hartikainen, H. Myxozoan Life Cycles: Practical Approaches and Insights. In Myxozoan Evolution, Ecology and Development; Okamura, B., Gruhl, A., Bartholomew, J.L., Eds.; Springer international Publishing, Switzerland, 2015; pp.175–198.
2. Okamura, B., Gruhl, A., Bartholomew, J.L. An Introduction to Myxozoan Evolution, Ecology and Development. In Myxozoan Evolution, Ecology and Development; Okamura, B., Gruhl, A., Bartholomew, J.L., Eds.; Springer international publishing, Switzerland, 2015; pp. 1–20.
3. Rocha, S., Rangel, L.F., Casal, G., Azevedo, C., Rodrigues, P. Santos, M.J. Involvement of sphaeractinomyxon in the life cycle of mugiliform-infecting Myxobolus (Cnidaria, Myxosporea) reveals high functionality of spore morphotypes in promoting transmission. Parasitology 2020, 147, 1320–1329.

Poster 5

Salmonella in Portuguese autochthonous hens breeds: a One Health concern

C. Miranda [1,2,*], V. Ribeiro [3], R. Dantas [3] and N. V. Brito [1,3]

[1] TOXRUN -Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL., Avenida Central de Gandra 1317, 4585-116 Gandra PRD, Portugal
[2] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Caparica, Portugal
[3] AMIBA - Associação dos Criadores de Bovinos de Raça Barrosã, 4730-260 Vila Verde, Portugal

* Correspondence: carla.miranda@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.70

Abstract

Background: The genus *Salmonella* is characterized as an enteric pathogen of mammals, reptiles, and birds and one of the most adapted environmental pathogens. These bacteria cause a high number of foodborne salmonellosis annually as a result of eating eggs, poultry and raw or undercooked meat contaminated with *Salmonella* [1,2]. Food-producing animals, in particular chickens, are considered reservoirs of this agent, associated with clinical illness and enormous risk to humans by the food chain. On the opposite, the local breeds in extensive or semi-extensive systems work to mitigate the impacts of intensive farming systems on food safety and public health, and enhance the rural economies. However, the studies on pathogen agents in local breeds are scarce [2,3]. **Objective:** The aim of this study was to determine the presence of *Salmonella* spp. in four Portuguese autochthonous chicken breeds in semi-extensive systems. **Methods:** A total of 87 samples of eggshells were obtained from 30 hens farms (2 to 4 samples/farm) of the following autochthonous breeds: "Pedrês Portuguesa" (n=22), "Amarela" (n=20), "Preta Lusitânica" (n=23) and "Branca" (n=22), in six different regions of Portugal during February of 2023. The *Salmonella* microbiological isolation was performed by the standard method recommended by ISO 6579:2017. Each sample was pre-enriched in Buffered peptone water, followed by incubation in Modified semi-solid Rappaport-Vassiliadis medium supplemented with novobiocin. From the culture obtained, selective solid media are inoculated: Chromagar Salmonella Plus agar®; Xylose lysine deoxycholate agar® and Salmonella-Sighella agar®. **Results:** From the four analyzed autochthonous hen breeds, no growth of characteristic *Salmonella* colonies was observed in the used selective solid media. **Conclusions:** To the best of our knowledge, this is the first study based on these autochthonous breeds, although the number of samples is limited. These preliminary results suggested that hen's eggs are not the most important vehicle of the infection by *Salmonella*, indicating a positive impact on animal health and public health.

Keywords: *Salmonella*; One Health; chicken; autochthonous breeds

Acknowledgments

This research was funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário, under the SALMYTH_GI2-CESPU_2022 project, entitled "Small scale authochtonous chicken productions - the salmonella myth". This work was financed by National Funds through FCT/MCTES – Portuguese Foundation for Science and Technology within the scope of the project UIDB/50006/2020 and UIDP/50006/2020.

References

1. Jibril, A.H.; Okeke, I.N.; Dalsgaard, A.; Kudirkiene, E.; Akinlabi, O.C.; Bello, M.B.; Olsen, J.E. Prevalence and risk factors of Salmonella in commercial poultry farms in Nigeria. *PloS one* **2020**, *15*(9), e0238190.
2. Brown, E. W.; Bell, R.; Zhang, G.; Timme, R.; Zheng, J.; Hammack, T.S.; Allard, M.W. Salmonella Genomics in Public Health and Food Safety. *EcoSal Plus* **2021**, *9*(2), eESP00082020.
3. Meira, M.; Afonso, I.M.; Casal, S.; Lopes, J.C.; Domingues, J.; Ribeiro, V.; Dantas, R.; Leite, J.V.; Brito, N. V. Carcass and Meat Quality Traits of Males and Females of the "*Branca*" Portuguese Autochthonous Chicken Breed. *Animals* **2022**, *12*(19), 2640.

Poster 6

In vitro antibacterial and cytotoxic activity of *Laurobasidium lauri* extracts

L. Silva [1], A. I. Oliveira [2], F. Moreira [2], R. Ferraz Oliveira [2] and C. Pinho [2,*]

[1] Escola Superior de Saúde, Instituto Politécnico do Porto, R. Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal
[2] Centro de Investigação em Saúde e Ambiente (CISA), Escola Superior de Saúde, Instituto Politécnico do Porto, R. Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal

* Correspondence: clp@ess.ipp.pt

https://doi.org/10.48797/sl.2023.72

Abstract

Background: Plants and mushrooms have been used as medicines for many years, as a source of antibiotics, antineoplastics, among others [1]. Research have been conducted on the medicinal uses of Portuguese plants, however, the therapeutic potentials of some of these plants used in traditional medicine, has remained unexploited. **Objective:** This study aims to evaluate antibacterial and cytotoxic activities, *in vitro*, of *Laurobasidium lauri*, a well-known fungus used in folk medicine on Madeira Island. **Methods:** An experimental study was performed using two extracts (aqueous and ethanolic 55% (V/V)) of the fungus isolated and in combination with three medicinal plants (*Parietaria judaica*, *Polygonum aviculare*, and *Peperomia galioides*). The antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus*, through disc diffusion and broth microdilution methods. Cytotoxicity was evaluated using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. **Results:** Regarding the disc diffusion method *E. coli* was not susceptible to any of the extracts, except for the antibiotic (41.0 ± 1.0 mm). However, *S. aureus*, when subjected to 10 mg/ml of the ethanolic extracts of *L. lauri* (isolated and in combination) exhibited an inhibition with a diameter of 16,3 ± 2,5 mm and 12,0 ± 1,7 mm, respectively, when compared to the control, ciprofloxacin (24,0±1,0 mm). Also, the ethanolic extract of the isolated fungus had the best value of minimum inhibitory concentration (MIC) for *S. aureus* (MIC = 0.078125 mg/mL). The ethanolic extract of the fungus in combination with medicinal plants showed greater cytotoxic action on lung cancer cells A549 (IC_{50} = 48.3±1.0 µg/mL). **Conclusions:** The fungus presented cytotoxic and antibacterial potential, and the results observed may be related to some bioactive compounds (e.g., costunolide and dehydrocostuslactone, two natural sesquiterpene lactones present in *Laurus* trees, where the fungus grows) [2]. However, more research is needed to confirm these biological activities and mechanisms of action.

Keywords: *Laurobasidium lauri*; antibacterial activity; cytotoxicity

Acknowledgments

This research received no external funding.

References

1. Friedman, M. Mushroom polysaccharides: chemistry and antiobesity, antidiabetes, anticancer, and antibiotic properties in cells, rodents, and humans. *Foods* **2016**, 5, 80.
2. Ferrari, B.; Castilho, P.; Tomi, F.; Rodrigues, A. I.; do Ceu Costa, M.; Casanova, J. Direct identification and quantitative determination of costunolide and dehydrocostuslactone in the fixed oil of Laurus novocanariensis by 13C-NMR spectroscopy. *Phytochem Anal* **2005**, 16, 104-107.

Poster 7

Far-UV-C radiation demonstrated germicidal activity against *Escherichia coli* and *Staphylococcus* sp.

M. Sousa [1,2,*], C. Sousa [1,2], M. Vassal [3], D. F. O. Braga [3] and M. Simões [1,2]

[1] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, Department of Chemical Engineering, University of Porto, 4200-465 Porto, Portugal
[2] ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal
[3] SpinnerDynamics, Lda., Rua da Junta de Freguesia 194, 4540-322 Escariz, Arouca, Portugal

* Correspondence: mfsousa@fe.up.pt

https://doi.org/10.48797/sl.2023.73

Abstract

Background: Bacteria are ubiquitous microorganisms with economic and public health impacts [1, 2]. Proper disinfection of public spaces minimizes bacterial contamination, spread, and associated diseases [3, 4]. Ultraviolet radiation (UV) is an effective and inexpensive approach for bacterial control and eradication [5-7]. **Objective:** This study aimed to evaluate the bactericidal potential of far-UV-C (222 nm) – an irradiation technology requiring scientific validation. **Methods:** The bactericidal effect of far-UV-C (from 104.6 µW/cm^2 to 918.0 µW/cm^2; 222 nm; 1 and 5 min), against adhered cells of *Escherichia coli* and *Staphylococcus* sp., was determined. Ultrasounds (for 1 min), as well as sodium hypochlorite, *N*-alkyl-*N*,*N*-dimethyl-*N*-benzyl-ammonium chloride, pentapotassium bis (peroxymonosulfate) bis(sulfate)), and perillyl alcohol (at sub-bactericidal concentrations for 5 min) were also tested. In addition, irradiation was combined with ultrasounds and each of the four compounds. Finally, the effect of the treatment UV + ultrasound + free chlorine was assessed. The effectiveness of each treatment was evaluated through the calculation of the percentage of reduction. **Results:** For 1 min of exposure to UV-C, percentages of reduction were between 42% and 94% for *E. coli*, while for *Staphylococcus* sp. these percentages ranged from 30% to 91%. For 5 min of exposure to radiation, percentages of reduction were from 79% to 100% for *E. coli* and were between 51% and 99% for *Staphylococcus* sp. Interestingly, the combinations were successful: UV + ultrasound showed synergism and the combinations of UV with each compound resulted in percentages of reduction equal to 100% for *E. coli* and higher than 96% for *Staphylococcus* sp. The triple combination resulted in a percentage of reduction of 100% for both bacteria. **Conclusions:** Far-UV-C reduces microbial contaminations successfully. In particular, combinatorial approaches were more effective than individual treatments, making these treatments adequate for disinfecting public spaces.

Keywords: bacterial contamination; public spaces disinfection; germicidal activity; UV-C light

Acknowledgments

This research was financially supported by LA/P/0045/2020 (ALiCE), UIDB/00511/2020, and UIDP/00511/2020 (LEPABE); funded by national funds through Germirrad-POCI-01-0247-FEDER-072237, by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES.

References

1. Orel, N; Fadeev, E; Klun, K; Ličer, M; Tinta, T; Turk, V. Bacterial Indicators Are Ubiquitous Members of Pelagic Microbiome in Anthropogenically Impacted Coastal Ecosystem. *Frontiers in Microbiology* **2022**, 12.
2. Cámara, M; Green, W; MacPhee, C.E.; Rakowska, P.D.; Raval, R; Richardson, M.C.; Slater-Jefferies, J; Steventon, K; Webb, J.S. Economic significance of biofilms: a multidisciplinary and cross-sectoral challenge. *npj Biofilms and Microbiomes* **2022**, 8(1):42.
3. Dhama, K; Patel, S.K.; Kumar, R; Masand, R; Rana, J; Yatoo, M.I.; Tiwari, R; Sharun, K; Mohapatra, R.K.; Natesan, S; *et al*. The role of disinfectants and sanitizers during COVID-19 pandemic: advantages and deleterious effects on humans and the environment. *Environ Sci Pollut Res Int* **2021**, 28(26), 34211-34228.
4. Khan, M.H.; Yadav, H. Sanitization During and After COVID-19 Pandemic: A Short Review. *Transactions of the Indian National Academy of Engineering* **2020**, 5(4), 617-627.
5. Rezaie, A; Leite, G.G.S.; Melmed, G.Y.; Mathur, R; Villanueva-Millan, M.J.; Parodi, G; Sin, J; Germano, J.F.; Morales, W; Weitsman, S; *et al*. Ultraviolet A light effectively reduces bacteria and viruses including coronavirus. *PLoS One* **2020**, 15(7):e0236199.
6. Chawla, A; Lobacz, A; Tarapata, J; Zulewska, J. UV Light Application as a Mean for Disinfection Applied in the Dairy Industry. *Applied Sciences* **2021**, 11(16):7285.
7. Yemmireddy, V; Adhikari, A; Moreira, J. Effect of ultraviolet light treatment on microbiological safety and quality of fresh produce: An overview. *Frontiers in Nutrition* **2022**, 9.

Poster 8

Survey of antimicrobial use during COVID-19 and environmental implications

C. De Mello-Sampayo [1,*], A. Almeida [2,3] and L. Meisel [4]

[1] Laboratory of Neuroinflammation, Signaling and Neuroregeneration, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal
[2] Centro de Investigação Vasco da Gama (CIVG), Departamento de Ciências Veterinárias, Escola Universitária Vasco da Gama (EUVG), Campus Universitário de Lordemão, 3020-210 Coimbra, Portugal
[3] Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), University of Coimbra, 3000-548 Coimbra, Portugal
[4] Laboratory of Systems Integration Pharmacology, Clinical and Regulatory Science, Research Institute for Medicines (iMED.Ulisboa), 1600-277 Lisbon, Portugal

* Correspondence: csampayo@ff.ulisboa.pt

https://doi.org/10.48797/sl.2023.74

Abstract

Background: The recent COVID-19 outbreak required the use of several antimicrobials in an attempt to find effective therapies. This COVID-19demanded use of several antimicrobials likely led not only to greater loads but also to a different pattern of antimicrobials in the environment. Clear understanding of the antimicrobial environmental threat requires frequent revaluation of the problem and disseminating it among relevant audiences. Thus, identifying the pandemic most used antimicrobials likely to pose environmental threat would be valuable [1,2]. **Methods:** The ambulatory and the hospitals consumption patterns of antimicrobials, during the COVID-19 pandemic (2020-2021) were compared to those of 2019. A predicted risk assessment screening approach based on exposure and hazard in the surface water was conducted, combining consumption and excretion rates endpoints in five different regions of Portugal. **Results:** Except for antimalarials, a negative consumption trend (from -2.5% to -15.0%) was observed for all antimicrobial groups over the study period. Among all antibiotics, antiviral and antimalarial used, 22 drugs showed an increased use with potential environmental concentrations compared to the pre-covid period. The microbiological risk quotient has been assessed and most of the 22 selected substances showed an elevated to moderate risk, with an impact on all regions, with flucloxacillin, piperacillin, tazobactam, meropenem, ceftriaxone, fosfomycin, metronidazole exhibiting the most significant potential to be selected for antibiotic resistance. **Conclusions:** Considering the present results, it is essential to promote monitoring of the positive risk-identified antimicrobials in the waste-surface water.

Keywords: antimicrobials; consumption; environmental risk; COVID-19

Acknowledgments

This research received no external funding.

References

1. Viana, P.; Meisel, L.; Lopes, A.; de Jesus, R.; Sarmento, G.; Duarte, S.; Sepodes, B.; Fernandes, A.; dos Santos, M. M. C.; Almeida, A.; et al. Identification of Antibiotics in Surface-Groundwater. A Tool towards the Ecopharmacovigilance Approach: A Portuguese Case-Study. Antibiotics, **2021**, 10 (888).
2. Silva, T. M.; Estrela, M.; Gomes, E. R.; Piñeiro-Lamas, M.; Figueiras, A.; Roque, F.; Herdeiro, M. T. The Impact of the COVID-19 Pandemic on Antibiotic Prescribing Trends in Outpatient Care: A Nationwide, Quasi-Experimental Approach. Antibiotics, **2021**, 10 (1040). https://doi.org/10.3390/antibiotics10091040.

Poster 9

Assessment of antimicrobial resistance throughout a wastewater treatment plant

O. Godinho [1,2], B. S. Diogo [1,2,3], S. C. Antunes [1,2], O. M. Lage [1,2] and S. Quinteira [1,4,5,*]

[1] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[2] CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal
[3] Instituto de Ciências Biomédicas Abel Salazar, ICBAS, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[4] BIOPOLIS/CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Rua Padre Armando Quintas, nº 7, 4485-661, Vairão, Portugal
[5] TOXRUN-Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL., Avenida Central de Gandra 1317, 4585-116 Gandra PRD, Portugal

* Correspondence: sandra.quinteira@fc.up.pt

https://doi.org/10.48797/sl.2023.75

Abstract

Background: As one of the leading worldwide causes of death, infections caused by antimicrobial resistant (AMR) pathogens, represent a global health crisis that can be tackled under the One Health approach [1]. Wastewater treatment plants (WWTP) have long been considered hotspots for transmission and selection of AMR genes and, as such, surveillance of AMR in WWTP is crucial [2, 3]. **Objective:** In this study we aimed to isolate AMR bacteria from different sites along a WWTP, and subsequently assess their phenotypic antimicrobial susceptibility profiles, and search for different AMR genetic determinants. **Methods:** Wastewater samples were collected from four sites within a WWTP for the isolation of *Enterobacteriaceae*, *Staphylococcus* spp., and *Enterococcus* spp. and inoculated on MacConkey Agar (MCA), Mannitol Salt Agar (MSA), and Slanetz Bartley Agar (SBA), with and without supplementation with antibiotics: imipenem or ciprofloxacin in MCA, while vancomycin in MSA and SBA. Isolate's phenotypic antimicrobial susceptibility profiles were determined by disk diffusion assay for several antibiotics (representative of different classes), according to CLSI guidelines [4]. Presence of integrons and resistance genes was assessed through PCR amplification. **Results:** A total of 50 bacterial isolates were obtained, of which 25 were affiliated with the genus *Enterococcus*. Ten isolates were affiliated with *Enterobacteriaceae*, and no isolate affiliated with *Staphylococcus spp.* were retrieved. Other isolates were affiliated with clinically relevant species such as *Pseudomonas aeruginosa* or *Aeromonas media*. Phenotypic resistances were observed in isolates from all wastewater samples, being the most common to tetracycline and ampicillin. Three isolates were resistant to three distinct classes of antimicrobials. Preliminary PCR screening results showed presence of tet(M) and class 1 integrons. **Conclusions:** This study reflects the importance of monitoring WWTP for presence of AMR genes, since AMR strains are found from the raw influent of the WWTP to the final effluent discharged into the environment.

Keywords: antimicrobial resistance; wastewater; One Health

Acknowledgments

This research was funded by Fundação para a Ciência e a Tecnologia, grants number (Ofélia Godinho – SFRH/BD/144289/2019, Bárbara Diogo – 2022.10505.BD, UIDB/04423/2020, UIDP/50027/2020 and UIDP/04423/2020).

References

1. Organization, W.H., WHO, FAO, and OIE unite in the fight against antimicrobial resistance. 2016: https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/tripartite/en/.
2. Michael, I., et al., Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res, 2013. 47(3): p. 957-95.
3. Milobedzka, A., et al., Monitoring antibiotic resistance genes in wastewater environments: The challenges of filling a gap in the One-Health cycle. J Hazard Mater, 2022. 424(Pt C): p. 127407.
4. (CLSI), C.a.L.S.I., Performance Standards for Antimicrobial Susceptibility Testing 30th edition, in Supplement M100. 2020: Pennsylvania, USA.

Poster 10

Siderophore-antimicrobial adjuvant conjugates as a strategy to fight antibacterial resistance

M. C. Almeida [1,2], P. M. da Costa [2,3], E. Sousa [1,2] and D. I. S. P. Resende [1,2,*]

[1] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge de Viterbo Ferreira 228, 4050-313, Porto, Portugal.
[2] CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal.
[3] ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.

* Correspondence: dresende@ff.up.pt

https://doi.org/10.48797/sl.2023.76

Abstract

Background: The levels of drug resistance to traditional antibiotics have been dramatically increasing constituting a threat to global health.[1] For this reason, the pursuit of alternative strategies to the discovery of novel antibacterial agents is a priority.[2] Emerging approaches include the development of target-directed compounds through the conjugation of antibiotics with moieties that will improve antibacterial activity.[2] A promising example is the conjugation of antibiotics with siderophores/siderophore mimetics able to highjack iron transport systems of bacteria acting as "Trojan Horses" [3, 4]. **Objective:** In this work, we aimed to synthesize conjugated molecules between siderophores/siderophore mimetics and antimicrobial adjuvants to obtain dual action compounds. Future goals also include the evaluation of the antibacterial activity and synergism potential of the compounds as well as the assessment of their effect on common resistant models. **Methods/Results:** Siderophore mimetics were synthesized through a variety of synthetic pathways. Then, two sequential coupling reactions were performed to connect the antibacterial adjuvant, the linker moiety and the siderophore or the siderophore mimetic. Structural elucidation of the compounds was obtained by nuclear magnetic resonance techniques (NMR). **Conclusions:** Siderophore-antimicrobial adjuvant conjugates described in this work were successfully synthetized and are expected to reveal potential as a novel approach to fight antimicrobial resistance.

Keywords: siderophores; antimicrobial adjuvants; drug resistance

Acknowledgments

This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry, CIIMAR) and project EXPL/CTA-AMB/0810/2021, under the PORTUGAL 2020 Partnership Agreement. Mariana C. Almeida acknowledges FCT for the individual PhD grant (2021.05224.BD) and Diana I. S. P. Resende for her individual researcher contract (2022.00379.CEECIND).

References

1. Nathan, C. Resisting antimicrobial resistance. *Nat. Rev. Microbiol.*, **2020**, *18*, 259-260.
2. Almeida, M. C.; da Costa, P. M.; Sousa, E.; Resende, D. I. S. P. Emerging Target-Directed Approaches for the Treatment and Diagnosis of Microbial Infections. *J. Med. Chem.* **2023**, *66*, 32-70.
3. Negash, K. H.; Norris, J. K. S.; Hodgkinson, J. T. Siderophore–antibiotic conjugate design: new drugs for bad bugs? *Molecules* **2019,** *24*, 3314.
4. Choi, J. J.; McCarthy, M. W. Cefiderocol: a novel siderophore cephalosporin. *Expert Opin Investig Drugs* **2018**, *27*, 193-197.

Poster 11

High occurrence of multidrug-resistant *Escherichia coli* from Holstein-Friesian cattle in Northern Portugal: a One Health challenge

S. Quinteira [1,2,3,*], A. B. Dias [4], L. Pinho [5], N. V. Brito [1], C. Campos [6,7], A. R. Freitas [1,8,9] and C. Miranda[1,10]

[1] TOXRUN-Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL., Avenida Central de Gandra 1317, 4585-116 Gandra PRD, Portugal
[2] BIOPOLIS/CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Rua Padre Armando Quintas, nº 7, 4485-661, Vairão, Portugal
[3] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[4] University Institute of Health Sciences, CESPU, Avenida Central de Gandra 1317, 4585-116 Gandra PRD, Portugal
[5] Department of Veterinary Clinics, Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal
[6] Instituto Português de Oncologia do Porto Francisco Gentil, 4200-072, Porto, Portugal
[7] Escola Superior de Saúde, Instituto Politécnico do Porto, 4200-072, Porto, Portugal
[8] UCIBIO/REQUIMTE, Applied Molecular Biosciences Unit, Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[9] Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Portugal
[10] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Caparica, Portugal

* Correspondence: sandra.quinteira@ipsn.cespu.pt

https://doi.org/10.48797/sl.2023.77

Abstract

Background: *Escherichia coli* is currently a leading pathogen for deaths associated with antimicrobial resistance (AMR) [1]. AMR related to food-producing animals is a public health risk requiring a One Health approach [1,2]. Animals are major reservoirs of antibiotic resistant *E. coli* which can easily reach humans through the food chain, direct contact, or the environment [3]. **Objective:** To analyze the AMR profiles of *E. coli* in fecal samples from Holstein-Friesian cattle, as information on AMR from dairy farming in Portugal is scarce. **Methods:** Samples (n=112) collected from 7 different farms at Northern Portugal were pooled during February-March 2023 based on age group (8 calves and 8 cows per farm). Characteristic *E. coli* colonies were selected from MacConkey Agar supplemented with or without antibiotics (4 µg/ml cefotaxime; 3 µg/ml colistin), for confirmation by MALDI-TOF mass spectrometry and antimicrobial susceptibility testing (AST), according to EUCAST/CLSI guidelines. ESBL phenotype were searched using the Double-Disk Synergy Test. **Results:** Presumptive *E. coli* isolates (n=110) representing different farming production systems were obtained and 51 representatives were identified by MALDI-TOF and tested by AST. From both calves and cows, 95% and 63% of the *E. coli* isolates exhibited resistance to ≥1 antimicrobial classes and multidrug resistance (MDR, resistance to ≥3 antimicrobial classes), respectively. Isolates were mostly resistant to gentamycin (88%), ampicillin (72%), amoxicillin plus clavulanic acid (63%), tetracycline (63%) and cefotaxime (53%). Only two isolates showed susceptibility two all tested antibiotics. ESBL activity was observed in 21% of *E. coli* isolates. All analyzed pools were classified as MDR. **Conclusions:** This is one of the first studies on AMR rates of *E. coli* from dairy cattle in Northern Portugal. It reveals a high prevalence of MDR *E. coli*, which is a worrying finding and emphasize the need of a multisectoral One Health approach to minimize its impact.

Keywords: One Health; antimicrobial resistance; livestock; *Escherichia coli*

Acknowledgments

This work was funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário, under the CORECattleGI2-CESPU-2022 project, entitled Prevalence of colistin-resistant *Enterobacteriaceae* on intensive and non-intensive cattle production systems. Work supported by National Funds through FCT - Fundação para a Ciência e a Tecnologia in the scope of the project UIDP/50027/2020, as well as UIDB/50006/2020 and UIDP/50006/2020.

References

1. Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, Shah T, Chaudhry TH, Rasool MH, Shahid A, Xueshan X and Baloch Z. Antibiotic Resistance: One Health one world outlook. *Front Cell Infect Microbiol* **2021**,11,771510.
2. Ma F, Xu S, Tang Z, Li Z, Zhang L. Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosafety and Health* **2021**,3,32-38.
3. Samtiya M, Matthews KR, Dhewa T, Puniya AK. Antimicrobial resistance in the food chain: trends, mechanisms, pathways, and possible regulation strategies. *Foods* **2022**,11,2966.

Poster 12

Vancomycin-resistant *Enterococcus faecium* circulating in a Portuguese hospital (2009-2021) challenges classical infection control

A. C. Almeida-Santos [1,2,*], B. Duarte [1,2], A. P. Tedim [3,4], M. D. Fernández-de-Bobadilla [5], L. M. Silva [6], H. Ramos [6], T. M. Coque [5], C. Novais [1,2], A. R. Freitas [1,2,7,ǂ] and L. Peixe [1,2,ǂ]

[1] UCIBIO/REQUIMTE, Applied Molecular Biosciences Unit, Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Portugal
[2] Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Portugal
[3] Grupo de Investigación Biomédica en Sepsis – BioSepsis, Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain.
[4] Centro de Investigación Biomédica en Red en Enfermedades Respiratorias (CIBERES, CB22/06/00035), Instituto de Salud Carlos III, Madrid, Spain
[5] Hospital Ramón y Cajal -IRYCIS, Madrid, Spain; CIBER in Infectious Diseases (CIBERINFEC), Madrid, Spain
[6] Serviço de Microbiologia, Centro Hospitalar do Porto, Porto, Portugal
[7] TOXRUN, Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[ǂ] these authors contributed equally to this work

* Correspondence: acfasantos@gmail.com

https://doi.org/10.48797/sl.2023.78

Abstract

Background: Vancomycin-resistant *Enterococcus faecium* (VREfm) prevalence greatly varies in European countries yet continues to rise [1]. We aimed to characterize clinical-VREfm from a local hospital (800-beds) during 2009-2021. **Methods:** VREfm [n=175; mostly from urine (40%)] were collected from medical-47% and surgical-32% wards. Antibiotic-resistance (ABR) and occurrence of *vanA/vanB*, plasmids [rep-pLG1/rep-pRUM/rep-Inc18(rep1/rep2)] and virulence genes were screened by disk-diffusion/broth-microdilution (EUCAST/CLSI) and PCR, respectively. Representative VREfm/year (n=81) were selected for MLST+WGS (Illumina-NovaSeq) and analysed through CGE-tools (homemade virulence and bacteriocins(bac) databases) and cgMLST/Ridom-SeqSphere^{+}. **Results:** All isolates were resistant to glycopeptides [98%-*vanA*;2%-*vanB*], ampicillin, ciprofloxacin, erythromycin [*erm*(B)/*msr*(C)], less to gentamicin (49%;*aac(6')-Ie-aph(2'')-Ia*), tetracycline [27%; *tet*(M)/*tet*(L)], quinupristin-dalfopristin (5%) and/or linezolid (2%;GT2576 mutation). VREfm were oligoclonal (until 7 STs/year), mostly clustering into ST117-31%, ST78-19% and ST80-12%. For each ST, different complex-types (CT) were detected each year, but some were identified in different wards from 3-months (ST412-CT258/ST117-CT4659) to 2-4-years (ST117-CT24/CT6602/CT6603; ST78-CT330). Sequenced VREfm showed slight overlap (ST117-CT24 and ST78-CT230) with other global Efm. Recent years (>2019) concentrated the majority of novel CTs, without common clones over time, and ST494 (associated with a German outbreak) was introduced. ABR and virulence patterns were similar throughout the years. Bac-profiles (13 patterns) were similar between common STs/CTs. One new bacteriocin was detected >2017, Bac43 increased over time and BacAS9 was exclusively detected in ST117. The typical VREfm plasmidome [RepA_N-pRUM/pLG1-like, Inc18, Rep3-pB82/pEF418, Rep_Trans-pEFNP1/pRI1] was observed across the years however, Rep-pRUM decreased. A novel RepA_N-replicase (69% nucleotide identity with pRUM) was identified in most VREfm during 2009-2021. *In silico* analysis showed its occurrence in ~100 Efm-genomes/GenBank from different countries after 2000. **Conclusion:** Current clinical VREfm display a strong oligoclonal nature. Nosocomial transmission events and/or import of VREfm from colonized patients may explain the long-term persistence of particular clones. The continuous flux between community and hospital clones may feed the emergence of new lineages/opportunities for adaption hampering classical infection control interventions.

Keywords: vancomycin resistance; *Enterococcus faecium*; clones; bacteriocins; nosocomial infections

Acknowledgments

This work received financial support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the exploratory project EXPL/SAU-INF/0261/2021.We acknowledge FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) for the national funds received through the projects UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences, project LA/P/0140/2020 of the Associate Laboratory, Institute for Health and Bioeconomy (i4HB). A.C.A.-S. gratefully acknowledges an UI/BD/151317/2021 fellowship granted by FCT/MCTES through national funds.

References

1. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual epidemiological report for **2021**. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2021. Stockholm: ECDC; 2022.

Poster 13

Where did you come from? – Characterization of pathogenic antibiotic-resistant *Escherichia coli* in recreational waters

L. Gomes [1,*], A. Machado [1,2] and A. A. Bordalo [1,2]

[1] ICBAS—UP, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[2] CIIMAR—UP, Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal

* Correspondence: up201704763@icbas.up.pt

https://doi.org/10.48797/sl.2023.22

Abstract

Background: *Escherichia coli* is a commensal bacterium present in the gastrointestinal tract of warm-blood animals, and therefore routinely used as a microbiological indicator of fecal contamination [1,2]. However, several strains may acquire virulence factors, becoming potentially pathogenic for humans and animals [1,3]. **Objective:** The current study underscores human exposure to pathogenic and antimicrobial resistant *E. coli* through recreational waters. **Methods:** Surface water samples were collected from estuarine and coastal beaches, as well as from treated wastewater. Detection and isolation were performed using selective and differential culture media. Presumptive isolates were confirmed by PCR approach. *E. coli* isolates were submitted to an extensive virulence gene screening. **Results:** The majority of the *E. coli* isolates belonged to the phylogenetic subgroup D1 (24%) or B1 (20%), indicating that animals were a relevant source of contamination. The isolates virulence profiles (n=272) showed that 35% were diarrheagenic *E. coli* (DEC). Among the pathotypes, enterotoxigenic *E. coli* had a prevalence of 12%, followed by enterohaemorrhagic *E. coli* with 10%. Avian pathogenic *E. coli* associated genes were detected, with a higher presence of the *fiuA* (44%), *ompT* (39%), and *iss* (36%) genes. The assessment of antibiotic susceptibility to 22 antibiotics of 9 different classes was carried using the Kirby-Bauer disc diffusion method. A total of 72% of isolates showed resistance to at least 3 different antibiotic classes. The highest percentage of resistance was showed to Erythromycin (98.5%), and Rifampicin (99.2%), whereas 100% susceptibility was found to Imipenem and Nitrofurantoin. **Conclusions:** The results confirmed the circulation of pathogenic *E. coli* and antimicrobial resistant strains in recreational waters. This study highlights the importance of monitoring additional parameters than those officially listed as fecal indicators, in order to improve risk management and guarantee the safe use of the resources.

Keywords: *Escherichia coli;* recreational water; antimicrobial resistance; source tracking; One Health

Acknowledgments

This research was funded by the ATLANTIDA project (NORTE-01-0145-FEDER-000040), supported by the Northern Portugal Regional Operational Program (NORTE2020), under the Portugal 2020 Partnership Agreement and by the European Regional Development Fund (FEDER).

References

1. Denamur, E.; Clermont, O.; Bonacorsi, S; Gordan, D. The population genetics of pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.* **2021**, *19(1)*, 37–54.
2. EU, European Parliament. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. *O. J.* **2006**, *4.3.2006 L 64/37*, 37–51.
3. Yu, D.; Banting, G.; Neumann, N. F. A review of the taxonomy, genetics, and biology of the genus *Escherichia* and the type species *Escherichia coli*. *Can. J. Microbiol.* **2021**, *67(8)*, 553–571.

Poster 14

Nasal colonization by *Staphylococcus aureus* in Health Sciences students and analysis of risk factors under a One Health perspective

L. M. Gomes-Sampaio [1,*], A. Cláudia-Ferreira [1], J. C. Prata [1,2], R. M. S. Azevedo [1], P. Pacheco [1], C. Campos [3,4], C. Novais [5,6], L. Peixe [5,6], R. J. Dinis-Oliveira [1,7,8], C. Coelho [9], C. Miranda [1,10], S. Quinteira [1,11,12] and A. R. Freitas [1,5,6]

[1] TOXRUN, Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal.
[2] Food Microbiology and Technology Laboratory, Department of Aquatic Production, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3] Instituto Português de Oncologia do Porto Francisco Gentil, 4200-072, Porto, Portugal
[4] Escola Superior de Saúde, Instituto Politécnico do Porto, 4200-072, Porto, Portugal
[5] UCIBIO/REQUIMTE, Applied Molecular Biosciences Unit, Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[6] Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Portugal
[7] UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[8] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[9] UNIPRO, Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal.
[10] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Caparica, Portugal
[11] BIOPOLIS/CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Rua Padre Armando Quintas, nº 7, 4485-661, Vairão, Portugal
[12] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, Porto, Portugal

* Correspondence: a29747@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.79

Abstract

Background: *Staphylococcus aureus* is the leading bacterial cause of death globally [1]. Nasal carriage of *S. aureus* increases the risk of invasive infections, including by methicillin-resistant *S. aureus* (MRSA) strains, but studies including Portuguese university students (PUS) are scarce. **Objective:** To analyse the prevalence of methicillin-susceptible *S. aureus* (MSSA) and MRSA among PUS enrolled in different courses/years (1st-4th) at IUCS-CESPU, characterize their antibiotic resistance profiles, and assess the potential risk factors. **Methods:** Swabs collected during March-December 2022 from anterior nares of 156 volunteers (median 22-years) were processed in mannitol-salt agar and, in parallel, enriched in brain-heart broth with NaCl 6.5% further plated onto ChromID® MRSA SMART. Typical colonies were stored for species identification (MALDITOF-MS) and antibiotic susceptibility testing (disk diffusion; EUCAST/CLSI guidelines). Each student completed a questionnaire comprising demographic/clinical/social parameters. Statistical analysis was conducted in IBM-SPSS Statistics 26 using binary logistic regression applying a backward stepwise (likelihood ratio) method, with α=0.05, selecting variables using Chi-square tests and Mann-Whitney U tests for which $p \leq 0.20$, >10 occurrences, not biologically correlated [2]. **Results:** Prevalence of MSSA and MRSA (cefoxitin screening) were 28.8% and 1.9%, respectively. From the 45 positive samples, 9% were multidrug-resistant, 38% were resistant to penicillin, 40% to erythromycin, 40% to clindamycin (inducible), 7% to cefoxitin, 2% to tetracycline, and 2% to rifampicin. Self-reported frequent contact with animals (OR=3.44, CI 95%: 1.10–10.66) were positively associated with *S. aureus*, while regular sports participation presented a negative association (OR=0.36, CI 95%: 0.17–0.77). Sports participation was not correlated with self-reported excellent health (χ^2=0.680, p=0.409). **Conclusions:** This is one of the first studies assessing MSSA/MRSA rates in PUS after the COVID-19 pandemics imposing higher self-protection/hygienization. While PUS-MSSA rates are similar to that previously observed, PUS-MRSA rates are slightly higher. Additional samples are being processed to explore future trends and other potential One Health factors influencing MSSA/MRSA colonization.

Keywords: *Staphylococcus aureus*; MRSA; university students; One Health

Acknowledgments

Research was partly funded by FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) for the national funds received through the projects UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences, project LA/P/0140/2020 of the Associate Laboratory, Institute for Health and Bioeconomy (i4HB).

References

1. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 2022, 400(10369), 2221-2248.
2. Ranganathan, P.; Pramesh, C.S.; Aggarwal, R. Common pitfalls in statistical analysis: Logistic regression. Perspect Clin Res 2017, 8(3), 148-151.

Poster 15

Metalloproteinases and cellular components in saliva from periodontitis patients: preliminary study

S. Aguilera [1,*], M. Relvas [2] and A. Viana da Costa [3]

[1] IUCS, CESPU, 4585-116 Gandra, Portugal
[2] UNIPRO - Oral Pathology and Rehabilitation Research Unit, IUCS, CESPU, 4585-116 Gandra, Portugal
[3] TOXRUN - Toxicology Research Unit, IUCS, CESPU, 4585-116 Gandra, Portugal

* Correspondence: a29640@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.80

Abstract

Background: Metalloproteinases (MMPs) are extracellular matrix macromolecules important in cellular development and morphogenesis, being capable of activating growth factors nearby, cell surface receptors, adhesion molecules and immune mediators [1]. Because type I collagen is the major component of the periodontal extracellular matrix, special attention is given to the role of collagenases, among them MMP-8, which is involved in the degradation of this matrix. Periodontitis (PD) is a destructive inflammatory disease of the supporting tissues of the teeth, affecting 11% of the world's population. Disease results from the interaction between the oral biofilm and the immune system's response [2]. Moreover, salivary MMP levels are associated with aggravation of periodontitis [3, 4], but no relation with which leukocytes populations yet. **Objective:** Our aim is to evaluate MMP-8 salivary levels and the corresponding leukocyte populations in patients with periodontitis stage I/II, stage III/IV and healthy controls. **Methods:** The study includes patients from the Dental Clinic appointments of the University Clinic of IUCS (7 healthy, 5 stage I/II, 7 stage III/IV). Collection of unstimulated saliva samples, followed by quantification of MMP-8 by ELISA and cellular recovery for leucocyte analysis by flow cytometry. **Results:** In a small sample of 19 individuals, studied so far, we observed that PD patients had higher MPP-8 levels than healthy controls. For cellular analysis, only 3 samples were studied, 3 healthy individuals. However, it has not yet been possible to establish any association with MMP-8 levels. **Conclusions:** In a near future, we expect our results to allow a possible association between MMP-8 salivary levels and periodontitis disease expression (stage versus grade). Flow cytometry analyses will allow for the evaluation of the leukocyte populations in saliva and their link with MMP-8 levels, in order to find a potential biomarker for periodontitis.

Keywords: saliva; MMP-8; periodontitis

Acknowledgments

This research was funded by GI2-CESPU, Project: AEPIAPD-GI-GI2-CESPU-2022.

References

1. Visse, R.; Nagase, H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* **2003**, *92*, 827-839.
2. Hajishengallis, G.; Korostoff, J.M. Revisiting the Page & Schroeder model: The good, the bad and the unknowns in the periodontal host response 40 years later. *Periodontology 2000* **2017**, *75*, 116-151.
3. Ebersole, J.L.; Schuster, J.L.; Stevens, J.; et al. Patterns of salivary analytes provide diagnostic capacity for distinguishing chronic adult periodontitis from health. *J Clin Immunol* **2013**, *33*, 271-279.
4. Zhang L; Xiue L; Yan H; Huang L. Salivary matrix metalloproteinase (MMP)-8 as a biomarker for periodontitis: A PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore). **2018**, 97(3): e9642.

Poster 16

Zebrafish as a model for biomedical research of acute kidney injury: an ultrastructural study

F. Fernandes-Pontes [1,*], A. D. Resende [2,3] and P. Silva [1,4]

[1] Laboratory of Histology and Embryology, Institute of Biomedical Sciences Abel Salazar (ICBAS), 4050-313 Porto, Portugal
[2] TOXRUN –Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[3] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, 4450-208 Matosinhos, Portugal
[4] iNOVA Media Lab, ICNOVA-NOVA Institute of Communication, NOVA School of Social Sciences and Humanities, Universidade NOVA de Lisboa, 1069-061 Lisbon, Portugal

* Correspondence: fernandaifpontes@gmail.com

https://doi.org/10.48797/sl.2023.81

Abstract

Background: Acute Kidney Injury (AKI) is a highly lethal health syndrome that results in a sudden loss of kidney function. It leads to nephron epithelial cells destruction and compromises urine output and the excretion of nitrogenous wastes, which are used as biomarkers [1,2]. In most cases, a decrease in mean arterial blood pressure occurs with further activation of other mechanisms to stabilize the blood volume and flow and to maintain body homeostasis [3,4]. **Objective:** Since a gentamicin-induced zebrafish (*Danio rerio*) model has already been used as an animal model for AKI, we aim to exhaustively describe this model regarding the histopathological and ultrastructural renal alterations, being one of the first studies providing this type of description [5]. **Methods:** Two groups of 15 zebrafish, gentamicin and control groups, were retroperitoneal injected and collected to light (four and six fish at 48 and 96h after injection, respectively) and electron microscopy (10 fish in each sampling time) analysis. In addition to the qualitative observation, we performed a semiquantitative analysis of the different renal tubules in the semithin and ultrathin sections. **Results:** We verified that the most affected cells are from the proximal tubule epithelium, mainly due to a damaged brush border that could lead to a defective absorption. Furthermore, using a semiquantitative approach, we verified a decrease in mitochondria quantity and size, while lysosomes and lipid droplets increased. **Conclusions:** This is the first study providing a detailed histological and ultrastructural description of the gentamicin-induced zebrafish kidney, which is essential for studying AKI and other kidney-related diseases. These results allow cellular and subcellular abnormalities identification, which could help the development of new treatments and therapies. This model is a valuable tool for studying kidney diseases, and a thorough understanding of kidney anatomy and physiology is critical for advancing our knowledge of these complex diseases.

Keywords: semiquantitative analysis; gentamicin; electron microscopy; histopathology

Acknowledgments

The authors acknowledge the financial support of ICBAS – U.Porto.

References

1. McCampbell, K.K.; Springer, K.N.; Wingert, R.A. Atlas of cellular dynamics during zebrafish adult kidney regeneration. *Stem Cells Int.* **2015**, *2015*, 1-19.
2. Morales, E.E.; Wingert, R. A. Zebrafish as a model of kidney disease. *Results Probl. Cell. Differ*. **2017**, *60*, 55-75.
3. Kellum, J.A.; Romagnani, P.; Ashuntantang, G.; Ronco, C.; Zarbock, A.; Anders, H.-J. Acute kidney injury. *Nat. Rev. Dis. Primers* **2021**, *7* (1), 1-17.
4. Patschan, D.; Müller, G.A. Acute kidney injury. *J. Inj. Violence Res.* **2015**, *7* (1), 19-26.
5. Kamei, C.N.; Liu, Y.; Drummond, I.A. Kidney regeneration in adult zebrafish by gentamicin induced injury. *JoVE* **2015**, *102*, 1-6.

Poster 17

Zebrafish as a valuable vertebrate model to study teratogenicity of pharmaceuticals and psychoactive substances

O. Ribeiro [1,2,3], L. Félix [1,4], C. Ribeiro [2,5], S. M. Monteiro [1,4] and J. S. Carrola [1,4,*]

[1] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[3] Centre for Functional Ecology, Department of Life Sciences Coimbra, Portugal
[4] Inov4Agro, Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, Vila Real, Portugal
[5] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal

* Correspondence: joao@utad.pt

https://doi.org/10.48797/sl.2023.82

Abstract

Background: The number of pharmaceuticals and psychoactive substances on the market increases each year, posing a need to understand their teratogenic effects on vertebrates. In the last two decades, there has been a growing interest in alternative vertebrate models, as part of the 3 R's principle (*Replacement, Reduction, and Refinement*) which led to the increased use of the zebrafish (*Danio rerio*) [1]. Besides, the easy observation of embryo development and the early developmental stages are not classified as experimental animals in the guidelines of the European Directive 2010/63/EU [2], which highlights the ethical advantages in teratogenic potential evaluation [3]. **Objective:** The study aimed to make a minireview of zebrafish as a model to assess the potential of teratogenicity of pharmaceuticals and psychoactive substances and analyse the major methodologies used to evaluate the malformations severity. **Methods:** The scientific literature search was done using *ScienceDirect* and *PubMed* search engine, looking for: zebrafish, *Danio rerio*, ecotoxicology, toxicology, malformations, teratogenicity, teratogen potential, and a selection of original papers and review was done. **Results:** This review confirm that zebrafish is a good model to make a pre-screening of the teratogenicity of pharmaceuticals and psychoactive substances, among others. However, the results reflect that several methodologies are used to assess the zebrafish malformations, showing a high variability and inconsistency in the evaluated endpoints and the nomenclature used. Additionally, each study uses its own scale of malformations severity, which can be evaluated using a quantitative method (different degrees of severity) or a binary method (present or absent). **Conclusions:** Zebrafish are a suitable alternative and complementary model to rodents (and other vertebrates) for massive screening of the potential teratogenic substances despite presenting differences for rodents. In addition, there is a need to standardize the classification and severity system for assessing malformations to improve the reproducibility and comparison between studies.

Keywords: Zebrafish; teratogenicity; psychoactive substances; animal model

Acknowledgments

This work was financially supported by Portuguese National Funds by Foundation for Science and Technology (FCT), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX) and UIDB/04033/2020 (CITAB/Inov4Agro). Ondina Ribeiro thanks the FCT for the PhD Grant 2022.12242.BD.

References

1. Bauer, B.; Mally, A.; Liedtke, D. Zebrafish embryos and larvae as alternative animal models for toxicity testing. *Int. J. Mol. Sci.* **2021**, *22*, 13417.
2. Commission, E., *Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. OJ L* **1986**, *358*, 1-28.
3. Raghunath, A., Perumal, E. Analysis of lethality and malformations during zebrafish (Danio rerio) development. In *Teratogenicity Testing. Methods in Molecular Biology*. Félix, L. Eds.; Humana Press, New York, 2018; Volume 1797, pp. 337-363.

Poster 18

Aminoxanthones as building blocks for the development of BINOL-based chemosensors

A. R. Rocha-Nunes [1,*], M. M. P. Borges [1], V. M. F. Gonçalves [1,2], M. E. Tiritan [1,3,4] and E. M. P. Silva [1,5]

[1] TOXRUN-Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[2] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal.
[3] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[4] CIIMAR—Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[5] LAQV, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

* Correspondence: a31605@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.83

Abstract

Background: The existence of D-amino acids (D-aa) in human brain is known for a long time, but only recent findings revealed they are neuro-active and can be therapeutically useful if detected in initial stages of Alzheimer Disease (AD) cognitive decline [1]. D-serine and D/L serine ratio in serum have been proposed as biomarkers for AD progression. Knowledge related to the role of D-aa in AD pathogenesis will facilitate novel therapeutic treatments and hence, improve patient's quality of life. An accurate, timely diagnosis and simple method is crucial to access early treatments and the xanthone scaffold has the desirable photophysical properties to be explore as fluorophores. **Objective:** Develop chiral xanthone derivative-based fluorophores as enantioselective probes for detection and quantification of D-aa and D/L aa ratios for AD diagnoses. **Methods:** To develop the new xanthone-based chiral derivatives, a strategy based in the synthesis of a xanthone containing a maleimide moiety (MX) obtained from an aminoxanthone (XNH2) was envisioned. The MX could then act as Michael acceptor for the reaction with 1,1'-bi-2-naphthol (BINOL), the chiral moiety that will allow the enantioselective interactions with aa. **Results:** The synthesis of XNH2 was achieved in two steps: nitration reaction of the xanthone with KNO_3 in H_2SO_4 followed by reduction with $SnCl_2$ in concentrated HCl [2,3]. The product was recrystallized in ethanol and allowed to react with maleic anhydride followed by reaction with sodium acetate in acetic anhydride to produce the MX derivative. Finally, the 1,4-addition of BINOL to MX was performed [2,3]. Structure elucidation and spectroscopic characterization of the xanthone-BINOL derivative are ongoing. **Conclusions:** The aminoxanthone was successfully employed for the synthesis of the maleimide intermediate that allowed the conjugation with the BINOL chiral moiety. The spectroscopy studies are in progress, but preliminary results revealed the potential use of the new molecule as a fluorescence probe.

Keywords: chirality; enantioselectivity; fluorescence; amino acids; neurodegenerative diseases

Acknowledgments

This research was funded by GI2-CESPU, grant number XANTAAL_GI2-CESPU_2022.

References

1. Seckler, J. M.; Lewis, S. J. Advances in D-amino acids in neurological research. *Int J Mol Sci* **2020**, *21*, 7325.
2. Grzelakowska, A.; Kolińska, J.; Zakłos-Szyda, M.; Sokołowska, J. Novel fluorescent probes for L-cysteine based on the xanthone skeleton. *J Photochem Photobiol A: Chem* **2020**, *387*, 112153.
3. Pace, T. C. S.; Monahan, S. L.; MacRae, A. I.; Kaila, M.; Bohne, C. Photophysics of aminoxanthone derivatives and their application as binding probes for DNA. *Photochem Photobiol* **2006**, 82, 78-87.

Poster 19

Synthesis of a rosamine-based lipid probe for the study of lipid phenomena in membrane biomimetic models

P. A. M. M. Varandas [1], A. J. A. Cobb [2], M. A. Segundo [1] and E. M. P. Silva [1,3,*]

[1] LAQV, REQUIMTE, Department of Chemistry Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo;
[2] Department of Chemistry, King's College London, 7 Trinity Street, London SE1 1DB, United Kingdom
[3] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal

* Correspondence: eduarda.silva@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.84

Abstract

Background: Rosamines are a class of fluorescent dyes, structurally related to rhodamines, that are commonly used as fluorophores in biological research since they have high quantum yields, photostability, and sensitivity to changes in their environment [1,2]. Rhodamines can also be derivatized to be coupled with lipids to create fluorescent lipid probes. These probes are useful for visualizing and studying various lipid-related processes, such as lipid metabolism, lipid transport, lipid signaling, and lipid dynamics in real-time [3]. **Objective:** The aim of the present work was to synthesize and characterize the spectroscopic fluorescence properties of lipids tagged with a rhodamine derivative for assessment of lipid phenomena using liposomes as biomimetic models. **Methods:** The synthetic strategy envisioned considers the preparation of a rosamine containing a (2-iodoacetyl)piperazin-1-yl moiety (RosPi) that would allow the bioconjugation with the amino group of 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphoethanolamine (POPE). **Results:** The synthesis of RosPi, based on previously described procedures, was achieved in seven steps in 12% overall yield [4,5]. Firstly, a microwave assisted cyclization of a tetrahydroxybenzophenone in water led to the synthesis of the xanthone intermediate (90% yield). Then, triflic anhydride was used to form the corresponding ditrifilxanthone (94% yield) which in turn reacted with the Boc-piperazine to deliver the aminated xanthene (65%). Nucleophilic addition of 1,3-dimethoxybenzene to the xanthone carbonyl (66%) followed by Boc-deprotection provided the unmasked bisammonium salt (74%). Finally, the salt was reacted with the 2-chloroacetyl chloride to give the diamide (67%) which was transformed into the final iodoacetamide derivative by reaction with sodium iodide (69%). Several attempts for the bioconjugation of RosPi with POPE were performed and purification and structural characterization of the product are in progress. **Conclusions:** The preparation of RosPi was successfully achieved. The structural characterization and spectroscopy studies of the bioconjugate POPE-RosPi are ongoing.

Keywords: xanthene; fluorescence; bioconjugation; sensor

Acknowledgments

This research was funded by PT national funds (FCT/MCTES, Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020 and UIDP/50006/2020. P. Varandas acknowledges FCT/MEC for his PhD grant (SFRH/BD/139714/2018).

References

1. Beija, M.; Afonso, C.A.M.; Martinho, J.M.G. Synthesis and applications of rhodamine derivatives as fluorescent probes. *Chem. Soc Rev* **2009**, 38, 2410-2433.
2. Lavis, L. D. Teaching old dyes new tricks: Biological probes built from fluoresceins. *Annu Rev Biochem* **2017**, *86*, 825-843.
3. Magalhães, N.; Simões, G.M.; Ramos, C.; Samelo, J.; Oliveira, A.C.; Filipe, H.A.L.; Ramalho, J.P.P.; Moreno, M.J.; Loura, L.M.S. Interactions between rhodamine dyes and model membrane systems - Insights from molecular dynamics simulations. *Molecules* **2022**, *27*, 1420.
4. Wu, L.; Burgess, K. Synthesis and spectroscopic properties of rosamines with cyclic amine substituents. *J Org Chem* **2008**, 73, 8711-8718.
5. Štacko, P.; Šebej, P.; Veetil, A.T.; Klán, P. Carbon-carbon bond cleavage in fluorescent pyronin analogues induced by yellow light. *Org Lett* **2012**, *14*, 4918-4921.

Poster 20

The ocean as a source of new anti-inflammatory and anti-pruritic molecules

M. S. Martins [1,2,*], S. Long [3], M. M. M. Pinto [1,2], I. F. Almeida [4,5], M. T. Cruz [6,7] and E. Sousa [1,2]

[1] CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[2] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[3] Department of Bioengineering, Royal University of Phnom Penh, Russian Federation Blvd (110), Phnom Penh, Cambodia
[4] UCIBIO—Applied Molecular Biosciences Unit, MedTech, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[5] Associate Laboratory i4HB, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[6] Center for Neuroscience and Cell Biology, Rua Larga - Faculdade de Medicina, POLO I Universidade de Coimbra, 3004-504 Coimbra, Portugal;
[7] Faculty of Pharmacy, University of Coimbra, Polo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

* Correspondence: up201605865@edu.ff.up.pt

https://doi.org/10.48797/sl.2023.85

Abstract

Background: Pruritus, the most common symptom of skin diseases, is considered a chronic condition when experienced for more than six weeks. Although the etiology of the symptom remains elusive, chronic pruritus has been associated with neurokinin 1 receptor (NK1R) and its agonist substance P [1]. Since pruritus and inflammation often go together, the development of compounds with dual activity, specifically anti-inflammatory and anti-pruritic, is an upcoming strategy [1,2]. **Objective:** The present work aimed the discovery of new molecules inspired in models from the sea, a source of unique chemical structures with anti-pruritic and anti-inflammatory activities. **Methods:** Seventy marine-inspired compounds were tested *in silico* regarding their binding affinity to NK1R and their pharmacokinetic properties evaluated using SwissADME software. *In vitro* molecules' cytotoxicity was evaluated in cells representative of the skin constitution, namely keratinocytes (HaCaT), macrophages (Raw 264.7), and fibroblasts (3T3). The anti-inflammatory properties were investigated in macrophages, by evaluating nitric oxide synthase (iNOS) protein levels (Western blot analysis), nitric oxide (NO) production (Griess assay) and NO scavenging potential using an *in chimico* assay. **Results:** The tested compounds demonstrated a high binding affinity for NK1R *in silico* and no relevant cytotoxicity *in vitro*. Some compounds were able to reduce inflammation through the decrease of the pro-inflammatory mediator NO, not because of their NO scavenging potential, but by decreasing iNOS protein levels, thus suggesting the blockade of pro-inflammatory signaling pathways upstream iNOS synthesis, namely the transcription factor NF-kB. Importantly, most tested marine-inspired compounds presented MW up to 500 and log P in the range 2.40-5.76 which favours good skin permeation. **Conclusions:** The ocean is a potential source of anti-inflammatory compounds and NK1R antagonists for the treatment of skin conditions associated with pruritus and inflame mation.

Keywords: chronic pruritus; inflammation; neurokinin 1 receptor; marine natural products; skin diseases

Acknowledgments

This research was supported by national funds through FCT - in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of UCIBIO, the project LA/P/0140/2020 of i4HB, UIDB/04423/2020 and UIDP/04423/2020 of Group of Marine Natural Products and Medicinal Chemistry—CIIMAR, the projects UIDB/04539/2020, UIDP/04539/2020 and LA/P/0058/2020 from CIBB and project ref. NORTE-01-0247-FEDER-047239, co-financed by FEDER, NORTE2020, Portugal2020. M. S. Martins acknowledges her Ph.D. research grant 2021.05964.BD fully supported by FCT.

References

1. Ikoma, A.; Steinhoff, M.; Ständer, S.; Yosipovitch, G.; Schmelz, M. The neurobiology of itch. *Nat Rev Neurosci* **2006**, 7, 535-547.
2. Wong, L.S.; Wu, T.; Lee, C.H. Inflammatory and noninflammatory itch: Implications in pathophysiology-directed treatments. *Int J Mol Sci* **2017**,18, 1485.

Poster 21

Alzheimer's disease: the neuroprotective potential of novel synthetic compounds targeting P-glycoprotein

A. R. Monteiro [1,2,*], M. Maia [3,4], M. Martins [3,4], E. Sousa [3,4], F. Remião [1,2] and R. Silva [1,2]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313 Porto, Portugal
[3] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[4] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Novo Edificio do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal

* Correspondence: up202002446@edu.ff.up.pt

https://doi.org/10.48797/sl.2023.88

Abstract

Background: Alzheimer's disease (AD) is a progressive neurological disorder characterized by cognitive decline associated, specifically, with the degeneration of cholinergic neurons [1]. Although the etiology of the disease remains elusive, with several pathophysiological mechanisms contributing to disease progression, two main pathological hallmarks are well described, namely the presence of 1) neurofibrillary tangles formed by unfolded protein aggregates (hyperphosphorylation of tau protein) and 2) extracellular aggregates of Aβ within the brain [2, 3]. **Objective:** The present work aimed to perform a screening of the potential neuroprotective effects of 21 novel small molecules in a cholinergic-differentiation model of neuronal cells using the SH-SY5Y neuroblastoma cell line. **Methods:** SH-SY5Y cells were differentiated into a cholinergic phenotype in response to treatment of at least 7 days with retinoic acid at a final concentration of 10 μM, under low serum conditions [4, 5]. The assays selected to evaluate the potential neuroprotective effects were chosen to replicate some of the emerging disease-related hallmarks, namely: metal ion dyshomeostasis, ferroptosis and impairments in Aβ clearance [modulation of P-glycoprotein (P-gp) activity] [3, 6]. **Results:** The results of this study highlighted the remarkable neuroprotective effects of the majority of compounds against iron (III)- and erastin-induced cytotoxicity, in addition to their ability to modulate P-gp activity. Furthermore, the P-gp activators (compounds I-VIII, XII and XXI) were evaluated in a cellular model of AD-like pathology of Aβ-induced cytotoxicity. The obtained results demonstrated the ability of compounds to protect the differentiated cells against the toxic stimulus, implicating the ATP-dependent efflux pump - P-gp - in the clearance of Aβ aggregates. **Conclusions**: Thus, this study reinforces the several efforts that have been made toward counteracting AD multifactorial nature by shifting the strategy into the design of P-gp activators for preventing, delaying or treating AD.

Keywords: Alzheimer's disease; amyloid-beta; P-glycoprotein; neuroprotection; disease-modifying drugs

Acknowledgments

This research was funded by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the projects UIDB/04423/2020, UIDP/04423/2020 (CIIMAR), UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

References

1. Hampel, H.; Mesulam, M.M.; Cuello, A.C.; *et al*. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* **2018**, 141, 1917-1933.
2. Du, X.; Wang, X.; Geng, M. Alzheimer's disease hypothesis and related therapies. *Transl Neurodegener* **2018**, 7, 1-7.
3. Savelieff, M.G.; Nam, G.; Kang, J.; Lee, H.J.; Lee, M.; Lim, M.H. Development of multifunctional molecules as potential therapeutic candidates for Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis in the last decade. *Chem Rev* **2019**, 119, 1221-1322.
4. Zhang, Y.P.; Brown, R.E.; Zhang, P.C.; Zhao, Y.T.; Ju, X.H.; Song, C. DHA, EPA and their combination at various ratios differently modulated Aβ25-35-induced neurotoxicity in SH-SY5Y cells. *Prostaglandins Leukot Essent Fat Acids* **2018**, 136, 85-94.
5. Medeiros, L.M.; De Bastiani, M.A.; De Rico, E.P.; Schonhofen, P. Cholinergic differentiation of human neuroblastoma SH-SY5Y cell line and its potential use as an in vitro model for Alzheimer's disease studies. *Mol Neurobiol* **2019**, 56, 7355-7367.
6. Chai, A.B.; Hartz, A.M.S.; Gao, X.; Yang, A.; Callaghan, R.; Gelissen, I.C. New evidence for p-gp-mediated export of amyloid-β peptides in molecular, blood-brain barrier and neuronal models. *Int J Mol Sci* **2021**, 22, 1-20.

Poster 22

Neuroprotective effects of mitochondria-targeted antioxidants in a Parkinson's disease *in vitro* model

I. Costa [1,2,*], S. Benfeito [3,*], V. Silva [1,2,3], F. Cagide [3], F. Borges [3], F. Remião [1,2] and R. Silva [1,2,*]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] CIQUP/IMS – Chemistry Research Unit of the University of Porto/Institute of Molecular Sciences, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

* Correspondence: inessilvacosta@hotmail.com; ester.benfeito@fc.up.pt; rsilva@ff.up.pt

https://doi.org/10.48797/sl.2023.89

Abstract

Background: Parkinson's disease (PD) is a neurodegenerative disease with early prominent death of dopaminergic neurons in the *substantia nigra pars compacta*, concurrently with Lewys body formation, iron accumulation, oxidative stress and ferroptosis[1–3]. Since there is no effective therapy capable of stopping/delaying disease progression, phenolic acids such as hydroxycinnamic and hydroxybenzoic acids (HCA and HBA, respectively), and their derivatives, are being extensively explored to target oxidative stress and iron overload, pathophysiological mechanisms involved in PD[4–6]. **Objective:** The main objective of this work was to evaluate, *in vitro*, the potential neuroprotective effects of a series of mitochondriotropic antioxidants (HCA and HBA derivatives) against iron overload and ferroptosis, mechanisms involved in PD pathophysiology. **Methods:** Differentiated SH-SY5Y cells were used as *in vitro* model and compounds (0–100 µM) cytotoxicity evaluated, 24h after exposure, by the neutral red uptake and resazurin reduction assays, to select non-cytotoxic concentrations. To evaluate the compounds' neuroprotective effects, two chemical aggressors were used, Fe(III) (500 and 1000 µM, to mimic iron overload) and erastin (20 and 40 µM, a ferroptosis inducer). The cytotoxicity of the chemical aggressors was evaluated by the NR uptake assay 24h after exposure to the oxidative insults in the presence and absence of the mitochondriotropic antioxidants (10 and 50 µM, non-cytotoxic concentrations). The potential neuroprotective effects against the combination of the two chemical aggressors was also evaluated [100 µM Fe(III) + 2 µM erastin]. **Results:** Ten of the 11 compounds significantly reduced Fe(III)-induced cell death, while seven compounds afforded a significant protection against erastin-induced cytotoxicity. Regarding the simultaneous exposure to Fe(III) and erastin (where only compounds that were neuroprotective against both the aggressors alone were tested), all the five compounds selected demonstrated significant neuroprotective effects. **Conclusions:** Although preliminary, these results clearly demonstrated the potential neuroprotective effects of these compounds, open a new perspective for PD treatment.

Keywords: Parkinson's disease; neuroprotection; mitochondriotropic antioxidants; disease-modifying drugs

Acknowledgments

This work was supported by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.This research was also funded by FEDER funds through the Operational Programme Competitiveness Factors-COMPETE and national funds by FCT – Foundation for Science and Technology (UIDB/00081/2020 (CIQUP), LA/P/0056/2020 (IMS), PTDC/MED-QUI/29164/2017 – POCI-01- 0145-FEDER-29164). Vera Silva (2020.05560.BD) and Sofia Benfeito fellowships/contracts are supported by FCT, FEDER/COMPETE and PT-OPENSCREEN-NORTE-01-0145-FEDER-085468.

References

1. Simon, D.K.; Tanner, C.M.; Brundin, P. Parkinson Disease Epidemiology, Pathology, Genetics, and Pathophysiology. *Clin Geriatr Med* **2020**, *36*, 1–12.
2. Lew, M. Overview of Parkinson's Disease. *Pharmacotherapy* **2007**, *27*, 155S-160S.
3. Schapira, A.H.; Jenner, P. Etiology and Pathogenesis of Parkinson's Disease *Mov. Disord* **2011**, *26*, 1049-1055.
4. Benfeito, S.; Oliveira, C.; Fernandes, C.; Cagide, F.; Teixeira, J.; Amorim, R.; Garrido, J.; Martins, C.; Sarmento, B.; Silva, R.; *et al*. Fine-tuning the neuroprotective and blood-brain barrier permeability profile of multi-target agents designed to prevent progressive mitochondrial dysfunction. *Eur J Med Chem* **2019**, *167*, 525–545.
5. Razzaghi-Asl, N.; Garrido, J.; Khazraei, H.; Borges, F.; Firuzi, O. Antioxidant properties of hydroxycinnamic acids: A review of structure-activity relationships. *Curr Med Chem* **2013**, *20*, 4436–4450.
6. Lee, D.-S.; Woo, J.-Y.; Ahn, C.-B.; Je, J.-Y. Chitosan-hydroxycinnamic acid conjugates: Preparation, antioxidant and antimicrobial activity. *Food Chem* **2014**, *148*, 97-104.

Poster 23

3-Hydroxypyridin-4-one based derivatives as promising neuroprotective agents for Parkinson's disease

V. Silva [1,2,3,*], S. Benfeito [3], C. Lima [3], D. Chavarria [3], T. Gonçalves [1,2], I. Costa [1,2], F. Remião [1,2], F. Borges [3] and R. Silva [1,2]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[2] UCIBIO - Applied Molecular Biosciences Unit, REQUIMTE, laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal
[3] CIQUP/IMS - Chemistry Research Unit of the University of Porto/Institute of Molecular Sciences, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

* Correspondence: veralssilva17@gmail.com

https://doi.org/10.48797/sl.2023.90

Abstract

Background: Parkinson's Disease (PD) is a multifactorial, complex and progressive neurodegenerative disease, characterized by the degeneration of dopaminergic neurons in the *substantia nigra pars compacta* [1,2]. Several pathophysiological mechanisms are involved in PD, namely Lewy bodies formation, mitochondrial dysfunction, neuroinflammation, oxidative stress and iron accumulation within the brain [3]. Iron triggers ferroptosis, a form of cell death characterized by uncontrolled lipid peroxidation, glutathione (GSH) depletion and decreased glutathione peroxidase 4 (GPx4) activity [4]. The drugs currently available to treat PD predominantly aim to relieve symptoms [5]. Therefore, there is an urgent demand for an effective treatment capable to stopping or slowing the disease progression. **Objective:** The main goal of this study was to evaluate, *in vitro*, the cytotoxicity and the neuroprotective effects of a small library of 3-hydroxypyridin-4-one based derivatives. **Methods:** Differentiated SH-SY5Y cells (dopaminergic phenotype) were used as *in vitro* model. The compounds' cytotoxicity was evaluated, 24h after exposure, by the neutral red uptake and resazurin reduction assays, to select the non-cytotoxic concentrations. To evaluate the potential neuroprotective effects of the compounds, cells were exposed for 24h to i) ferric nitrilotriacetate (FeNTA), a ferric (Fe^{3+}) iron aggressor, ii) *tert*-butyl hydroperoxide (*t*-BHP), an organic peroxide capable of inducing oxidative stress-mediated cell death, or iii) (1S,3R)-RSL3 (RSL3), a ferroptosis inducer that acts by inhibiting GPX4. The exposures were performed in the absence or presence of the test compounds. **Results:** In general, the compounds showed a safe cytotoxic profile for concentrations up to 5 μM. Noteworthy, several derivatives showed significant, concentration-dependent protective effects against *t*-BHP, FeNTA and RSL3, highlighting their promising neuroprotective effects. **Conclusions:** In conclusion, the 3-hydroxypyridin-4-one based derivatives demonstrated a multitarget mode of action, highlighting their potential as promising neuroprotective agents for PD treatment.

Keywords: Parkinson's disease; dopamine; ferroptosis; oxidative stress; hydroxypyridin-4-ones

Acknowledgments

This research was funded by national funds from FCT (FCT, Portugal), I.P., in the scope of the projects: a) UIDP/04378/2020 and *UIDB/04378/2020*, of the UCIBIO, b) LA/P/0140/2020 of the i4HB, c) UIDB/00081/2020 of the CIQUP) and d) LA/P/0056/2020 of the IMS, and by FEDER funds PTDC/MED-QUI/29164/2017 – POCI-01- 0145-FEDER-29164, EXPL/BIA-BQM/0492/2021. V. Silva was supported by a PhD fellowship (2020.05560.BD) from FCT. S. Benfeito, C. Lima and D. Chavarria fellowships/contracts are supported by FCT, FEDER/COMPETE and PT-OPENSCREEN-NORTE-01-0145-FEDER-085468.

References

1. Simon, D.K.; Tanner, C.M.; Brundin, P. Parkinson's disease epidemiology, pathology, genetics, and pathophysiology. *Clin Geriatr Med* **2020**, 36, 1-12.
2. Raza C.; Anjum R.; Shakeel, N.U.A. Parkinson's disease: Mechanisms, translational models and management strategies. *Life Sciences* **2019**, 226, 77-90.
3. Kouli, A.; Torsney, K.M.; Kuan, W.L. Parkinson's disease: etiology, neuropathology, and pathogenesis. In *Parkinson's Disease: Pathogenesis and Clinical Aspects*, 2nd ed.; Stoker, T.B., Greenland, J.C., Eds.; Publisher: Brisbane, Australia, **2018**; Section 1.
4. Moreau, C.; Duce, A.J.; Rascol, O.; Devedjian, J.C.; Berg, D.; Dexter, D.; Cabantchik, Z.I.; Bush, A.I.; Devos, D. Iron as a therapeutic target for Parkinson's disease. *Mov Disord* **2018**, 33, 568-574.
5. Dong, J.; Yanhua, C.; Song, L.; Weidong, L. Current pharmaceutical treatments and alternative therapies of Parkinson's disease. *Curr Neuropharmacol* **2016**, 14, 339-355.

Poster 24

New 8-hydroxyquinoline derivatives as promising therapeutic approaches targeting neurodegeneration

R. Marques-Oliveira [1,2,*], A. R. Alfenim [3], P. Soares [3], F. Borges [3], F. Remião [1,2], C. Fernandes [3] and R. Silva [1,2]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] CIQUP/IMS - Chemistry Research Unit, Institute of Molecular Sciences, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

* Correspondence: ritoliveira.m@gmail.com

https://doi.org/10.48797/sl.2023.92

Abstract

Background: Alzheimer's disease, Parkinson's disease, and Amyotrophic lateral sclerosis are recognized as the most prevalent neurodegenerative diseases (NDs), presenting a huge burden for society. These diseases share common pathophysiological mechanisms, such oxidative stress, dysfunction in iron metabolism, ferroptosis, and protein misfolding [1-4]. Given their powerful metal chelating and antioxidant properties, 8-hydroxyquinoline (8-HQs) derivatives have emerged as attractive therapeutic approaches for the development of innovative therapies for NDs [5]. **Objective:** To assess, *in vitro*, the neuroprotective effects of 12 newly synthesized 8-HQs with iron chelation and radical scavenging capacity, using differentiated neuronal SH-SY5Y cells as an *in vitro* model. **Methods:** The cytotoxicity of 8-HQs was initially evaluated using the MTT reduction and neutral red uptake assays, 24 hours after exposure, to select non-cytotoxic concentrations. The neuroprotective effects of the 8-HQs against the cytotoxicity induced by iron (III), erastin (a ferroptosis inducer), or by the combination of the two aggressors, were then evaluated. Their capacity to decrease *tert*-butyl hydroperoxide (*t*-BHP)-induced cytotoxicity was also investigated, aiming to elucidate the potential of the novel 8-HQs derivatives to counteract oxidative stress. The most promising 8-HQs were also tested for their ability to protect against the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+), which is frequently used to mimic Parkinson's disease in *in vitro* models [6]. **Results:** Some of the 8-HQs significantly protected SH-SY5Y cells against the cytotoxicity induced by iron (III), erastin, or by the combined effects iron (III) + erastin. Moreover, several 8-HQs also remarkably reduced the cytotoxic effects induced by both *t*-BHP and MPP^+. **Conclusions:** The 8-HQs showed outstanding neuroprotective properties against the harmful effects induced by distinct chemical aggressors, highlighting their potential to become effective disease-modifying agents for counteracting neurodegeneration.

Keywords: neurodegenerative diseases; 8-hydroxyquinolines; SH-SY5Y cells; oxidative stress; ferroptosis

Acknowledgments

This project was supported by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 895144. This work was also supported by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.This research was also funded by FEDER funds through the Operational Programme Competitiveness Factors-COMPETE and national funds by FCT – Foundation for Science and Technology (UIDB/00081/2020 (CIQUP), LA/P/0056/2020 (IMS), PTDC/MED-QUI/29164/2017 – POCI-01-0145-FEDER-29164). C.Fernandes (2021.04016.CEECIND) thanks FCT by his stimulus to scientific employment.

References

1. Checkoway, H.; Lundin, J.I.; Kelada, S.N.; Neurodegenerative diseases. *IARC Sci Publ* **2011**, 407-419.
2. Lane, C.A.; Hardy, J.; Schott, J.M. Alzheimer's disease. *Eur J Neurol* **2018**, 25, 59-70.
3. Kalia, L.V.; Lang, A. E. Parkinson's disease. *Lancet* **2015**, 386, 896-912.
4. Feldman, E.L.; Goutman, S. A.; Petri, S.; Mazzini, L.; Savelieff, M.G.; Shaw, P.J.; *et al*. Amyotrophic lateral sclerosis. *Lancet*, **2022**, 400, 1363-80.
5. Lim, C.K.; Kalinowski, D.S.; Richardson, D.R. Protection against hydrogen peroxide-mediated cytotoxicity in Friedreich's ataxia fibroblasts using novel iron chelators of the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone class. *Mol Pharmacol* **2008**, 74, 225-235.
6. Xicoy, H.; Wieringa, B.; Martens, G.J.M. The SH-SY5Y cell line in Parkinson's disease research: a systematic review. *Mol Neurodegener* **2017**, 12, 10

Poster 25

Cell microarray as a powerful tool to accelerate cancer research: focus on drug screening

M. Nunes [1,2,*,ǂ], D. Nunes [1,3,ǂ], A. Costa [4,ǂ] and S. Ricardo [1,3,5]

[1] Differentiation and Cancer Group, Institute for Research and Innovation in Health (i3S) of the University of Porto, 4200-135 Porto, Portugal
[2] Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal
[3] TOXRUN, Toxicology Research Unit, University Institute of Health Sciences, Polytechnic and University Cooperative (CESPU), 485-116 Gandra, Portugal
[4] Instituto Universitário de Ciências da Saúde (IUCS), Polytechnic and University Cooperative (CESPU), 485-116 Gandra, Portugal
[5] Faculty of Medicine from University of Porto (FMUP), 4200-319 Porto, Portugal
[ǂ] these authors contributed equally to this work

* Correspondence: mnunes@i3s.up.pt

https://doi.org/10.48797/sl.2023.93

Abstract

Background: Designing reliable *in vitro* assays is crucial to attain impactful results in oncology research. Cell Microarray (CMA) has become a key tool to accelerate cancer research as many samples can be evaluated at the same time in a single slide, allowing the evaluation of many prognostics, diagnostics, and therapy response biomarkers. Previously, we described a model of drug combination using antineoplastic and repurposed drugs to find alternative oncology regimens [1,2]. **Objective:** The purpose of this study is to adapt a histology-based method to evaluate the changes on biomarkers expression during drug efficacy tests and explore the mechanisms of therapy resistance in a chip-like tool such as a CMA. **Methods:** Two chemoresistant ovarian cancer models, i.e., OVCAR8 (Carboplatin-resistant) and OVCAR8 PTX RP (Carboplatin and Paclitaxel-resistant) cell lines [3] were incubated for 48 h with Carboplatin and Paclitaxel, alone and combined with repurposed drugs (Pitavastatin, Metformin and Ivermectin), using their half-maximal inhibitory concentration (IC_{50}) previously assessed. Next, we collected the cells subjected to all assay conditions and constructed a CMA, gathering all the conditions in a single paraffin block [3-5]. **Results:** CMA have the potential to accelerate cancer research studies since it allows the evaluation and comparison of a variety of cell culture conditions, such as several cell lines, time-points, and different therapeutical conditions, on a single microscope slide [4,5]. **Conclusions:** This approach represents a rapid and cost-effective screening tool to accelerate anti-cancer drug efficacy studies, allowing the discovery of biomarkers capable to predict therapy responses and to unveil the mechanism of action of new drugs, repurposed drugs, and drug combinations.

Keywords: biomarkers; cancer research; cell microarray; cell cultures; drug screening

Acknowledgments

This work was developed at i3S/IPATIMUP, an Associate Laboratory of the Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), and partially supported by Fundação para a Ciência e a Tecnologia (FCT)/MCTES. This research was supported by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant number "OVCARTEST_GI2-CESPU_2022". Mariana Nunes and Diana Nunes acknowledge FCT/MCTES and União Europeia (UE) for financial support through a PhD fellowship (2020.04720.BD and 2021.05081.BD, respectively) co-sponsored by Fundo Social Europeu (FSE) through Programa Operacional Regional Norte (Norte 2020).

References

1. Nunes, M.; Duarte, D.; Vale, N.; Ricardo, S. Pitavastatin and Ivermectin Enhance the Efficacy of Paclitaxel in Chemoresistant High-Grade Serous Carcinoma. *Cancers (Basel)* **2022**, *14*.
2. Nunes, M.; Duarte, D.; Vale, N.; Ricardo, S. The Antineoplastic Effect of Carboplatin Is Potentiated by Combination with Pitavastatin or Metformin in a Chemoresistant High-Grade Serous Carcinoma Cell Line. *Int J Mol Sci* **2022**, *24*.
3. Nunes, M.; Silva, P.M.A.; Coelho, R.; Pinto, C.; Resende, A.; Bousbaa, H.; Almeida, G.M.; Ricardo, S. Generation of Two Paclitaxel-Resistant High-Grade Serous Carcinoma Cell Lines With Increased Expression of P-Glycoprotein. *Front Oncol* **2021**, *11*, 752127.
4. Duarte, D.; Nunes, M.; Ricardo, S.; Vale, N. Combination of Antimalarial and CNS Drugs with Antineoplastic Agents in MCF-7 Breast and HT-29 Colon Cancer Cells: Biosafety Evaluation and Mechanism of Action. *Biomolecules* **2022**, *12*.
5. Magalhaes, A.C.; Ricardo, S.; Moreira, A.C.; Nunes, M.; Tavares, M.; Pinto, R.J.; Gomes, M.S.; Pereira, L. InfectionCMA: A Cell MicroArray Approach for Efficient Biomarker Screening in In Vitro Infection Assays. *Pathogens* **2022**, *11*.

Poster 26

Biological evaluation of new diarylpentanoid analogues for antitumor activity

E. Castro [1,*], J. Moreira [2,3], P. M. A. Silva [1,4], L. Saraiva [5], M. Pinto [2,3], H. Bousbaa [1] and H. Cidade [2,3]

[1] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal
[2] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[4] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[5] LAQV/REQUIMTE, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

* Correspondence: a11230@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.26

Abstract

Background: Cancer is associated with high mortality rates and its incidence worldwide is increasing significantly [1]. Several therapeutic strategies have been used in cancer therapy such as microtubule-targeting agents [2]. However, these drugs are highly toxic and are associated with high tumor resistance, making their long-term use unfeasible [3,4]. Therefore, new small molecules that can overcome the disadvantages associated with the drugs currently in use in the clinic are needed. We previously reported the *in vitro* growth inhibitory effect of the diarylpentanoid BP-M345 on human cancer cells, as well as its cellular mechanism of action [5]. Here, BP-M345 analogues have been synthetized in a goal to improve the antimitotic/antitumor efficacy of the original BP-M345. **Objective:** The present study aimed to characterize BP-M345 analogues regarding their cytotoxic activity and mechanism of action, focusing on their potential as antimitotic agent. **Methods:** A sulforhodamine B assay was used to determine the GI_{50} of BP-M345 analogues in different cancer cell lines. To evaluate the antimitotic activity, the mitotic index was determined. In addition, lung cancer cells were exposed to compounds, for 24- or 48 hours, and the consequence on spindle morphology, cellular proliferation and cell death were evaluated using the following assays: immunofluorescence, colony-formation assay, and flow cytometry, respectively. Time-lapse microscopy imaging was also performed to follow in real time the cell fate of the compound-treated cells. **Results:** BP-M345 analogues showed potent growth inhibition activity on cancer cells and exhibited a potent antimitotic activity. Mechanistically, BP-M345 analogues induced perturbation of the mitotic spindles through microtubule instability. Consequently, treated cells exhibit defects in chromosome congression during mitosis, which induced a prolonged spindle assembly checkpoint-dependent mitotic arrest, followed by apoptosis. **Conclusions:** BP-M345 analogues have been shown to be highly potent antimitotic agents, more effective than the original BP-M345 leading to massive cancer cell death.

Keywords: diarylpentanoid; anticancer; antimitotics; apoptosis; cancer

Acknowledgments

This research was funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário Crl [Grants Ref. Flav4Tumor-GI2-CESPU-2022; Ref. SGA4Cancer-GI2-CESPU-2022", and Ref. upPTXovcar-GI2-CESPU-2022]. This research was partially supported by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry, CIIMAR), through national funds provided by the FCT and ERDF, within the framework of the program PT2020 and the project PTDC/SAUPUB/28736/2017. Joana Moreira acknowledges her PhD grant (SFRH/BD/135852/2018).

References

1. Siegel, R.L.; Miller, K.D.; Wagle, N.S.; Jemal, A. Cancer statistics. *CA Cancer J Clin* 2023, 73, 17-48.
2. Wood, K.W.; Cornwell, W.D.; Jackson, J.R. Past and future of the mitotic spindle as an oncology target. *Curr Opin Pharmacol* **2001**, 1, 370.377.
3. Novais, P.; Silva, P.M.A.; Amorim, I.; Bousbaa, H. Second-generation antimitotics in cancer clinical trials. *Pharmaceutics* **2021**, 13, 1011.
4. Henriques, A.C.; Ribeiro, D.; Pedrosa, J.; Sarmento, B.; Silva, P.M.; Bousbaa, H. Mitosis inhibitors in anticancer therapy: When blocking the exit becomes a solution. *Cancer Lett* **2019**, 440:64-81.
5. Novais, P.; Silva, P.; Moreira, J.; Palmeira, A.; Amorim, I.; Pinto, M.; Bousbaa, H. BP-M345, a new diarylpentanoid with promising antimitotic activity. *Molecules* **2021**, 26, 7139.

Poster 27

Tumor aggregates from ovarian cancer patients ascitic fluid present low caspase-3 expression

C. Batista-Pinto [1,2,*], A. D. Resende [1,2], R. Andrade [1], F. Garcez [1,3], V. Ferreira [4], C. Lobo [4], P. Monteiro [4], M. H. Abreu [5,6], C. Bartosch [4,6,7] and S. Ricardo [1,2,8,9]

[1] IUCS, Instituto Universitário de Ciências da Saúde, CESPU, CRL, 485-116 Gandra, Portugal
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[3] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[4] IPOP – Instituto Português de Oncologia do Porto, Departamento de Patologia, 4200-162 Porto, Portugal
[5] IPOP – Instituto Português de Oncologia do Porto, Serviço de Oncologia Médica, 4200-162 Porto, Portugal
[6] Porto Comprehensive Cancer Center Raquel Seruca, 4200-162 Porto, Portugal
[7] Grupo Biologia do Cancro & Epigenética, Centro de Investigação do IPOP, 4200-162 Porto, Portugal
[8] i3S – Institute for Research and Innovation in Health, University of Porto, Differentiation and Cancer Group, 420-135 Porto, Portugal
[9] FMUP – Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

* Correspondence: carlam.pinto@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.94

Abstract

Background: Ascites is observed in ovarian cancer advanced stages because of the inflammatory process caused by tumor cells invasion of the peritoneal cavity [1,2]. In the ascitic fluid microenvironment, tumor cells can be found isolated or forming aggregates, being key mediators of transceolomic metastization [1,2]. *In vitro* multicellular spheroids show anoikis resistance presenting high survival levels when compared to isolated tumor cells [3]. The viability of these tumor cells is crucial for the establishment of patient-derived organoids (PDOs) that constitute a valuable preclinical platform for drug testing [3]. **Objective:** This study aims to evaluate the tumor cells apoptotic levels (single cells and aggregates) in the ascitic fluid of ovarian cancer patients. **Methods:** We evaluated 23 cytologic samples from ovarian cancer patients with ascites admitted at IPOPorto under a project approved by IPOPorto ethics committee (CES.092R1/019). Standard histologic processing was performed on the formalin-fixed and Histogel™ embedded ascitic fluid. For the apoptotic cell detection an immunohistochemistry technique with anti-caspase-3 antibody was applied and evaluated by microscopy. **Results:** During standardization, the ideal primary antibody concentration and incubation time were set, as also the antigenic retrieval procedure was optimized. We included a positive control to validate the technique in each run. Our results show that, in most of the samples, cellular aggregates were negative for caspase-3 expression (>75% of the cells) but some positivity was observed in isolated tumor cells. **Conclusions:** The evaluation of caspase-3 expression by immunohistochemistry proved to be a reliable methodology to evaluate the apoptotic levels in cytology samples. In general, tumor cells within aggregates showed high viability levels, whereas some isolated tumor cells presented caspase-3 expression, which indicate they are undergoing an apoptotic process. The tumor aggregates high viability in these samples is a good indicator that the establishment of PDOs from these tumor cells will be successful.

Keywords: ascites; tumor aggregates; apoptosis; immunohistochemistry; patient-derived organoids

Acknowledgments

This work was developed in association with i3S/IPATIMUP, an Associate Laboratory of the Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), and partially supported by Fundação para a Ciência e Tecnologia (FCT)/MCTES. This research was supported by CESPU, grant number "OVCARTEST_GI2-CESPU_2022" and TOXRUN.

References

1. Nunes, D.; Ricardo, S. Ovarian Cancer Ascites as a Liquid Tumor Microenvironment. In *Ovarian Cancer* [Internet]; Lele S., editor; Brisbane (AU): Exon Publications, 2022; Chapter 3.
2. Al Habyan, S.; Kalos, C.; Szymborski, J.; McCaffrey, L. Multicellular detachment generates metastatic spheroids during intra-abdominal dissemination in epithelial ovarian cancer. *Oncogene* **2018**, *37*(37), 5127–5135.
3. Nunes, M.; Ricardo, S. Chemoresistance in Ovarian Cancer: The Role of Malignant Ascites. In *Ovarian Cancer* [Internet]; Lele S., editor; Brisbane (AU): Exon Publications, 2022; Chapter 2.

Poster 28

Co-inhibition of MPS-1 with BCL-2 family inhibitors enhances lung cancer cell killing in 2D and 3D culture systems

B. Pinto [1,2,*], P. M. A. Silva [1,3], B. Sarmento [4], J. Carvalho-Tavares [2,*] and H. Bousbaa [1,5]

[1] Unidade de Investigação em Patologia e Reabilitação Oral (UNIPRO), Instituto Universitário de Ciências da Saúde (IUCS), CESPU, Rua Central de Gandra 1317, 4585-116, Gandra, Portugal
[2] Universidade Federal de Minas Gerais (UFMG), Instituto de Ciências Biológicas, Departamento de Fisiologia e Biofísica, Av. Pres. Antônio Carlos 6627, 31270-901, Belo Horizonte, Brasil
[3] Unidade de Investigação em Toxicologia (TOXRUN), Instituto Universitário de Ciências da Saúde (IUCS), CESPU, Rua Central de Gandra, 1317, 4585-116, Gandra, Portugal
[4] i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135, Porto, Portugal.
[5] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n 4450-208, Matosinhos, Portugal

* Correspondence: barbara_fernandes_pinto@hotmail.com

https://doi.org/10.48797/sl.2023.24

Abstract

Background: Lung cancer is the leading cause of cancer death worldwide, posing a significant public health challenge [1]. Currently available therapies, when administered as monotherapy, have limited efficacy, high toxicity, and can lead to increased tumor resistance. Overexpression of MPS-1, a protein kinase involved in mitosis, has been observed in various types of tumors. Its inhibition is associated with aberrant chromosome segregation, leading to cell death. Also, overexpression of anti-apoptotic proteins from the BCL-2 family has been reported in different cancer types, and inhibiting them can enhance cancer cell killing [2,3]. **Objective:** To assess the antitumor potential of combining an MPS-1 inhibitor with a BCL-2 family inhibitor, in both 2D and 3D lung cancer cells (A549). **Methods:** MPS-1 mRNA and protein levels were assessed by qRT-PCR and Western blot, respectively. In 2D cultures, the compounds cytotoxic activity was evaluated by MTT assay. The effects of the combination (antagonistic/additive/synergistic effects) were determined using the Combenefit software. The cell death was evaluated by TUNEL method and by flow cytometry (annexin V/propidium iodide). To assess the antiproliferative activity, the colony formation assay was performed. In 3D cultures, spheroid viability and apoptosis were determined by CellTiter-Glo assay and annexin V/ propidium iodide labeling, respectively. **Results:** Our results demonstrated that MPS-1 mRNA and protein levels were increased in A549 cells. Co-treatment of 2D cultures with the MPS-1 inhibitor and the BCL-2 family inhibitor resulted in various synergistic points. The combination with the lowest pharmacological concentrations inhibited cancer cell proliferation, and induced cell death by apoptosis. The results were confirmed in a 3D spheroid model. **Conclusions:** Cancer cell killing activity of the MPS-1 inhibitor is enhanced when combined with the BCL-2 family inhibitor, both in 2D and 3D cultures.

Keywords: MPS-1 inhibitor; BCL-2 family inhibitor; antimitotics; antitumoral activity; combination therapy

Acknowledgments

This research was funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário Crl (Grants Ref. AntiMitoSphere_APSFCT_IINFACTS_2021; Ref. Flav4Tumor-GI2-CESPU-2022; Ref. SGA4Cancer-GI2-CESPU-2022", and Ref. upPTXovcar-GI2-CESPU-2022) to Hassan Bousbaa and Patrícia Silva. Bárbara Pinto gratefully acknowledges CESPU for financial support (BD/CBAS/CESPU/01/2020), and Fundação para a Ciência e a Tecnologia (FCT) for financial support (2022.09451.BD).

References

1. Duma, N.; Santana-Davila, R.; Molina, J.R. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clin Proc* **2019**, *94*, 1623-1640.
2. Henriques, A.C.; Ribeiro, D.; Pedrosa, J.; Sarmento, B.; Silva, P.M.A.; Bousbaa, H. Mitosis inhibitors in anticancer therapy: When blocking the exit becomes a solution. *Cancer Lett* **2019**, *440*, 64-81.
3. Pinto, B.; Novais, P.; Henriques, A.C.; Carvalho-Tavares, J.; Silva, P.M.A.; Bousbaa, H. Navitoclax Enhances the Therapeutic Effects of PLK1 Targeting on Lung Cancer Cells in 2D and 3D Culture Systems. *Pharmaceutics* **2022**, *14*, 1209.

Poster 29

Metabolic reprogramming of sunitinib- and pazopanib-resistant renal cell carcinoma cells: a metabolomics approach

F. Amaro [1,2,*], M. Carvalho [1,2,3,4], M. L. Bastos [1,2], P. Guedes de Pinho [1,2] and J. Pinto [1,2]

[1] Associate Laboratory i4HB, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[2] UCIBIO-REQUIMTE, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] FP-I3ID, FP-BHS, University Fernando Pessoa, Porto, Portugal
[4] Faculty of Health Sciences, University Fernando Pessoa, Porto, Portugal

* Correspondence: up201608003@up.pt

https://doi.org/10.48797/sl.2023.27

Abstract

Background: Tyrosine kinase inhibitors (TKIs), such as sunitinib and pazopanib, changed the therapeutic landscape of metastatic renal cell carcinoma (RCC) [1,2]. However, TKIs resistance and disease progression within one year have been observed even in patients who initially respond to treatment [3]. Hence, understanding the metabolic mechanisms associated with TKIs resistance is of utmost importance to reverse this issue and improve RCC treatment guidelines. **Objective:** This work applied a metabolomics approach to investigate the metabolic dysregulations underlying sunitinib and pazopanib resistance in a metastatic RCC cell line (Caki-1). **Methods:** Caki-1 cell line was continuously (6 months) exposed to increasing concentrations of sunitinib and pazopanib to induce resistance. Resistance was confirmed through the MTT assay by a 4.9- and 2.8-fold increase in the IC_{50} values of sunitinib and pazopanib-resistant cells compared with the parental cells, respectively. In the metabolomics assay, eight independent passages were considered for TKI-resistant and parental cells. Intracellular and extracellular metabolites were analyzed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. Statistical analysis comprised multivariate and univariate methods, and biological interpretation was performed through pathway analysis. **Results:** TKIs-resistant cells revealed a common reprogramming in the amino acid, glycerophospholipid, and nicotinate and nicotinamide metabolisms. Sunitinib-resistant cells were also characterized by an enhanced cellular antioxidant capacity supported by a significant increase in the intracellular levels of glutathione and myo-inositol, and a significantly higher uptake of glutamine. On the other hand, pazopanib-resistant cells exhibited marked changes in several metabolites (e.g., glucose, lactate, pyruvate, acetate, succinate, fumarate) participating in energy metabolism. **Conclusions:** Our findings demonstrate for the first time a distinct pattern of metabolic alterations associated with sunitinib and pazopanib resistance in metastatic RCC cells. Targeting these dysregulations may constitute a promising strategy to restore cell sensitivity to treatment with these TKIs.

Keywords: renal cell carcinoma; tyrosine kinase inhibitors resistance; *in vitro* metabolomics

Acknowledgments

This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. Filipa Amaro thanks FCT for her PhD scholarship UI/BD/151313/2021.

References

1. Escudier, B., Porta, C., Schmidinger, M., Rioux-Leclercq, N., Bex, A., Khoo, V., Grunwald, V., Gillessen, S., Horwich, A., ESMO Guidelines Committee. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up dagger. *Ann. Oncol*. **2019**, 30, 706–720.
2. Motzer, R.J., Hutson, T.E., Cella, D., Reeves, J., Hawkins, R., Guo, J., Nathan, P., Staehler, M., de Souza, P., Merchan, J.R., et al. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. *N. Engl. J. Med.* **2013**, 369, 722–731.
3. Buczek, M., Escudier, B., Bartnik, E., Szczylik, C., Czarnecka, A. Resistance to tyrosine kinase inhibitors in clear cell renal cell carcinoma: from the patient's bed to molecular mechanisms. *Biochim Biophys Acta*. **2014**, 1845, 31-41.

Poster 30

Co-inhibition of mitotic kinesins and kinases with BCL-2 family inhibitors enhances cytotoxicity of oral cancer cells

J. P. N. Silva [1,*], L. Monteiro [1], P. M. A. Silva [1,2] and H. Bousbaa [1]

[1] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal

* Correspondence: joaosilva_06@hotmail.com

https://doi.org/10.48797/sl.2023.25

Abstract

Background: Head and neck cancer is the seventh most prevalent cancer worldwide and it includes oral cancer [1]. Approximately 90% of all oral cancers are oral squamous cell carcinomas [2,3]. Despite their promising preclinical results, the inhibitors of mitotic kinesins and kinases failed in clinical trials, probably because they are not too effective in inducing apoptosis when used in monotherapy [4,5]. Thus, apoptotic pathway in cell treated with mitotic kinesin and kinase inhibitors may be a strategy to improve the effectiveness of these antimitotic agents. **Objective:** Kinesin spindle protein (KSP) is involved in chromosome segregation and its inhibition results in the formation of monopolar spindles, inducing a mitotic delay. Aurora B is involved in correcting errors in chromosome attachment to the mitotic spindle, and its inhibition leads to mitotic exit with chromosome missegregation. The objective of this study is to assess the antitumor potential of combining a KSP or an Aurora B inhibitor with a BCL-2 family inhibitor in oral cancer. **Methods:** To evaluate the cytotoxic activity of the inhibitors, the IC_{50} was determined by the MTT assay. Using the Combenefit software, the combinations corresponding to the lowest concentration of the drugs that resulted in the greatest cytotoxic effect were selected. Cell death was assessed by flow cytometry, using annexinV/PI staining. Cell fate after combination treatment was monitored and characterized by time-lapse microscopy. **Results:** Both anti-KSP/anti-BCL2 and anti-Aurora B/anti-BCL2 combinations showed synergistic effects with increased cytotoxic activity. The anti-KSP/anti-BCL2 combination showed an exacerbation of apoptosis during mitotic arrest, while the anti-Aurora B/anti-BCL2 combination led to increased postmitotic death. **Conclusions:** Our data demonstrate that the combination of a BCL2 family inhibitor with either a KSP or an Aurora B inhibitor may be potentially useful as treatment strategies against oral cancer.

Keywords: antimitotics; BCL-2 family inhibitor; apoptosis; combination therapy; oral cancer

Acknowledgments

This research was funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário Crl [Grants Ref. Flav4Tumor-GI2-CESPU-2022; Ref. SGA4Cancer-GI2-CESPU-2022; and Ref. upPTXovcar-GI2-CESPU-2022]. JS gratefully acknowledges CESPU for financial support (BD/CBAS/CESPU/01/2021).

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA. Cancer J. Clin.* **2021**, *71*, 209–249.
2. Nandini, D.B.; Rao, R.S.; Hosmani, J.; Khan, S.; Patil, S.; Awan, K.H. Novel Therapies in the Management of Oral Cancer: An Update. *Disease-a-Month* **2020**, *66*, 101036.
3. Thomson, P.J. Perspectives on Oral Squamous Cell Carcinoma Prevention—Proliferation, Position, Progression and Prediction. *J. Oral Pathol. Med.* **2018**, *47*, 803–807.
4. Henriques, A.C.; Ribeiro, D.; Pedrosa, J.; Sarmento, B.; Silva, P.M.A.; Bousbaa, H. Mitosis Inhibitors in Anticancer Therapy: When Blocking the Exit Becomes a Solution. *Cancer Lett.* **2019**, *440–441*, 64–81.
5. Novais, P.; Silva, P.M.A.; Amorim, I.; Bousbaa, H. Second-Generation Antimitotics in Cancer Clinical Trials. *Pharmaceutics* **2021**, *13*, 1011.

Poster 31

Impact of cannabidiol on viability of normal and tumorigenic human kidney cells: are the effects serum-dependent?

D. Sousa [1,2,*], F. Amaro [3,4], A. M. Araújo [5] and M. Carvalho [1,2,3,4]

[1] FP-I3ID, FP-BHS, University Fernando Pessoa, Porto, Portugal
[2] Faculty of Health Sciences, University Fernando Pessoa, Porto, Portugal
[3] Associate Laboratory i4HB, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4] UCIBIO/REQUIMTE, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[5] LAQV/REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Porto, Portugal

* Correspondence: 39766@ufp.edu.pt

https://doi.org/10.48797/sl.2023.29

Abstract

Background: Cannabidiol (CBD), the main non-psychotropic cannabinoid of *Cannabis sativa*, has several pharmacological actions with therapeutic potential, including antitumor effects [1]; however, its effect on renal cell carcinoma (RCC) is unknown. Considering that recent research suggests that cell culture conditions, particularly the presence of serum in culture medium, may modulate the cannabinoids' antitumor effects [2], the potential influence of this growth supplement on the response to CBD should be explored. **Objective:** To fill this gap, we investigated the sensitivity of human kidney cells to CBD in different growth conditions. **Methods:** CBD's cytotoxic profile was assessed in non-tumoral (HK-2) and tumoral (Caki-1 and 769-P) human renal cell lines, using 0% or 5% FBS. The MTT assay was performed at different time-points (24 and 48h) after cells were exposed to a wide range of CBD concentrations (1-100 µM). **Results:** CBD induced a concentration-dependent decrease in cell viability across all cell lines and conditions. After 24h at 5% FBS, it was found that HK-2 and Caki-1 cells were the most sensitive to CBD toxicity, followed by 769-P cells (IC_{50} values were respectively 14.5, 14.8, and 20.0 µM). In a serum-free medium, after 24h, IC_{50} values markedly decreased (5.2, 7.6, and 6.8 µM for HK-2, Caki-1 and 769-P cells, respectively; $p<0.004$ *vs*. 5% FBS), demonstrating that FBS has a large impact on cellular sensitivity to CBD. IC_{50} values obtained for 48h were similar since no time-dependent effect was observed ($p>0.05$). **Conclusions:** Our findings support that CBD has *in vitro* anticancer potential against RCC cells, with greater cytotoxic efficacy in the absence of serum. However, CBD cytotoxicity was not selective for tumoral cells, which may be a significant limitation to its safe use in clinical practice. More research is being conducted to investigate the cell death signaling pathways activated by CBD in each cell line.

Keywords: cannabidiol; renal cell carcinoma; antitumoral activity; serum

Acknowledgments

This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. Filipa Amaro thanks FCT for her PhD scholarship UI/BD/151313/2021.

References

1. Seltzer, E.S.; Watters, A.K. Cannabidiol (CBD) as a Promising Anti-Cancer Drug. *Cancers* **2020**, *12*, 3203.
2. Sainz-Cort, A.; Müller-Sánchez, C. Anti-proliferative and cytotoxic effect of cannabidiol on human cancer cell lines in presence of serum. *BMC Res Notes* **2020**, *13*, 389.

Poster 32

Optimization of a protocol for isolation of immune cells from European seabass (*Dicentrarchus labrax*)

D. Salazar-Gutierrez [1,2], M. Ferreira [1,2], V. Sousa [1,2], L. M. P. Valente [1,2], A. Correia [2,3,4] and S. Gomes [1,2,*]

[1] CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, 4450-208 Matosinhos, Portugal
[2] ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, 4050-313 Porto, Portugal
[3] i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135 Porto, Portugal
[4] IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal

* Correspondence: sagomes@icbas.up.pt

https://doi.org/10.48797/sl.2023.30

Abstract

Background: As one of the most important food production sectors worldwide, aquaculture is now invested in developing tools to study fish immunity. Fish health relies heavily on hematopoietic organs (head kidney), but also on intestine. The wide knowledge-gap of the fish immune system coupled with the lack of cell lines to perform *in vitro* studies poses a barrier to the development of efficient tools to enhance fish immunity. **Objective:** We optimized the protocol for isolation of immune cells from the head kidney (HK) and posterior intestine (PI) of European seabass, to be used as an *in vitro* tool to test the impact of environmental contaminants on the fish immune system. **Methods:** HK and PI of European seabass were collected following the methodology of Park et al. [1]. Tissues were pushed through cell strainers. Cell suspensions were layered on a Percoll gradient of 34%/51% (HK) and 25%/75% (PI) and the intermediate band was thereafter collected. Isolated cells were resuspended in L-15^{+} media with 10% FBS and allowed to adhere to cell culture dishes for 24-h at 23ºC. Adherent cells were detached with PBS 7 mM EDTA on ice. Isolated cells were analyzed by flow cytometry followed by sorting, using lectins WGA and LEL as markers. The cells' morphology was assessed using cytospinning followed by diff quick staining. **Results:** We observed a heterogeneous cell population in HK and PI. A high number of leucocytes was isolated from HK (lymphocytes, neutrophils, and monocytes); while in PI the number of leucocytes was scarce. WGA and LEL markers failed to distinguish the cell populations. Based on their morphology, the adherent cells seemed to be enriched in monocytes and/or macrophages. **Conclusions:** The development of protocols for isolation and culturing of immune cells can be used as a steppingstone for further studies on fish immunity.

Keywords: European seabass; immune cells; *in vitro* tools

Acknowledgments

This research received funding from FCT - Foundation for Science and Technology within the scope of BASSinCELLS (2022.08942.PTDC) UIDB/04423/2020 and a PhD grant to Mariana Ferreira (SFRH/BD/144843/2019). Financial support from the Fund for Bilateral Relations, from the EEA Grants, in the framework of the bilateral initiative - FBR_OC1_124 – AquaCell. The partners in this project were CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental (Portugal as Initiative Promoter) and Nord University-Faculty of Biosciences and Aquaculture (Norway as Partner) is also acknowledged.

References

1. Park, Y., Zhang, Q., Wiegertjes, G. F., Fernandes, J. M. O., Kiron, V. Adherent Intestinal Cells From Atlantic Salmon Show Phagocytic Ability and Express Macrophage-Specific Genes. *Front. Cell Dev. Biol.* **2020**, 8, 580848.

Poster 33

Tea and herbal infusions consumption in Portugal: consumer behaviors

A. C. Sousa [1,*], C. Ribeiro [1], V. M. F. Gonçalves [1,2], I. Pádua [1,3] and S. Leal [1,4]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[2] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal
[3] Instituto de Saúde Pública da Universidade do Porto (ISPUP), Rua das Taipas 135, 4050-600 Porto, Portugal
[4] CINTESIS@RISE, MEDCIDS, Faculty of Medicine, University of Porto, Rua Dr. Plácido da Costa, 4200-450 Porto, Portugal

* Correspondence: a23452@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.31

Abstract

Background: Tea and herbal infusions (THIs) consumption is recognized for its beneficial effects, which are attributed to the antioxidant properties of phenolic compounds [1–3].The wide diversity of THIs available in market and the absence or failure in quality control can expose the population to health risks [3]. Moreover, uncontrolled consumption may cause toxicity or lack of the expected benefits. Therefore, an understanding of the THIs consumption and consumer habits will allow adjustments in risk-benefit assessment for vulnerable population. **Objective:** The main aim was to understand THI consumption in Portugal. **Methods:** A web-based survey was developed and applied (January-February 2023), using Google forms® platform, after the Ethical Committee approval. Sociodemographic data, type of THIs consumed and brewing were collected. Descriptive statistics was performed using JASP 0.16.1.0. **Results:** A total of 720 participants completed the online survey, of which 47.2% declared drinking THIs ≥2 cups/week (medium consumers) and 17.8% consumed ≥2 cups/day (heavy consumers). In the latter, the majority were female (87.5%), of which 51.8% aged 40-60 years. In heavy THI consumers group, 10% were older than 61 years. Among the heavy and medium consumers, herbal Infusions, such as citronella (42.1%) and chamomile (35.7%), were most popular than green tea consumption (22.3%). For THI preparation, mostly of these consumers reported the use of tap water (82%) and bags (56%), but 41% reported the use either bags or leaves. Over 16% of heavy and medium consumers use more product quantity than recommended. Approximately 86% of the heavy and medium consumers indicated that, after boiling, let THIs rest for 5-10 minutes and, 78% removed bag/leave before drinking. **Conclusions:** Herbal infusions consumption was more frequent than green tea and heavy consumers were mainly older adults. This study contributes with relevant results for future risk–benefit assessments of THIs consumption available in the Portuguese market.

Keywords: tea and infusions consumption; web-based survey; consumer choice; beverages preparation

Acknowledgments

This work is supported by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant "SPAinT-GI2-CESPU-2022".

References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) *et al*. Scientific opinion on the safety of green tea catechins. *EFSA J* **2018**, 16, e05239.
2. EFSA Panel on Contaminants in the Food Chain (CONTAM) *et al*. Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. *EFSA J* **2017**, *15*, e04908.
3. Assunção, R.; Twarużek, M.; Kosicki, R.; Viegas, C.; Viegas, S. Drinking Green Tea: Despite the Risks Due to Mycotoxins, Is It Possible to Increase the Associated Health Benefits?. *Toxins* **2021**, *13*, 119.

Poster 34

Can culinary processing impact the lipid content and fatty acid profile of turbot (*Scophthalmus maximus*)?

A. Fiorin [1,2,3,*], A. Marques [3], A. F. Silva [3], C. Castro [4], P. Moreira [2,5,6], P. Padrão [2,5,6] and L. M. P. Valente [3,7]

[1] Faculdade de Ciências da Universidade do Porto, Campo Alegre, S/N, Porto, Portugal
[2] Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Campo Alegre, 823, Porto, Portugal
[3] Centro Interdisciplinar de Investigação Marinha e Ambiental, Av. General Norton de Matos, S/N, Matosinhos, Portugal
[4] Flatlantic, Rua do Aceiro, S/N, Coimbra, Portugal
[5] Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal
[6] EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Portugal
[7] ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Rua de Jorge Viterbo Ferreira, 228, Porto, Portugal

* Correspondence: aninha_fiorin@yahoo.com.br

https://doi.org/10.48797/sl.2023.32

Abstract

Background: Fish are the main source of omega-3 long-chain polyunsaturated fatty acids (PUFAs), such as EPA and DHA, which are known to be beneficial for human health [1,2]. However, thermal culinary processing may cause lipid oxidation and reduce its nutritional value [1]. **Objective:** This study aims to compare the lipid nutritional value of turbot (*Scophthalmus maximus)* muscle before and after culinary processing, including traditional oven and grilled, with and without olive oil. **Methods:** The impact of muscle location (ventral and dorsal), and the skin presence on the fish's lipid content and fatty acid (FA) profile was also evaluated. Total lipids were quantified gravimetrically by Folch methodology and the FA was analyzed by gas chromatography. **Results:** Results showed that skin presence did not significantly impact lipid content or FA profile, but the ventral muscle had a significantly higher total lipid content than the dorsal muscle, irrespective of the culinary processing or skin presence. The dorsal section had significantly higher relative concentrations of C20:4n-6, DHA and EPA+DHA, while the ventral section was richer in C14, C16:1n-7, C18:3n-3, and n-6/n-3 ratio. But the deposition of EPA (mg/100g), was greater in the ventral muscle section. The fatty acid profile of processed samples did not differ significantly from fresh muscle. However, the application of extra virgin olive oil increased MUFA and decreased PUFA levels in processed samples. A significant interaction between culinary processing, skin and oil presence was observed in n-3, n-6/n-3. The n-3 FA were significantly higher in muscle processed without oil resulting in the lowest n-6/n-3 ratio. **Conclusions:** Overall, the nutritional value of all muscle samples is very high irrespectively of culinary processing and all provided consumers with more than 500mg of EPA+DHA/100g, complying with EFSA recommended daily intakes to prevent cardiovascular diseases in adults [3].

Keywords: culinary processing; fatty acid profile; PUFA; lipid oxidation; turbot

Acknowledgments

This work is a result of the project OmegaPeixe (POCI-01-0247-FEDER-069748), supported by Operational Program for Competitiveness and Internationalization (COMPETE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and by FCT – Foundation for Science and Technology to CIIMAR (UIDB/04423/2020, UIDP/04423/2020). PC was supported through an ARDITI grant funded by the M1420-09-5369-0002 project.

References

1. Biandolino, F.; Parlapiano, I.; Denti, G.; Di Nardo, V.; Prato, E. Effect of Different Cooking Methods on Lipid Content and Fatty Acid Profiles of Mytilus galloprovincialis. *Foods* **2021**, *10*, 416.
2. Moradi, Y.; Bakar, J.; Motalebi, A.A.; Syed Muhamad, S.H.; Che Man, Y. A Review on fish lipid: Composition and changes during cooking methods. *J. Aquat. Food Prod. Technol.*, **2011,** *20*, 379-390.
3. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J* **2010**, *8*, 1461.

Poster 35

In vitro toxicity assessment of firefighters' breathable air collected on polyurethane foams in human lung epithelial cells

M. J. Bessa [1], J. Teixeira [2], C. Delerue-Matos [2], B. Sarmento [1,3], F. Rodrigues [2] and M. Oliveira [2,*]

[1] UNIPRO – Unidade de Investigação em Patologia e Reabilitação Oral, Instituto Universitário de Ciências da Saúde (IUCS), CESPU, Gandra, Portugal
[2] REQUIMTE/LAQV, Instituto Superior de Engenharia, Instituto Politécnico do Porto, Porto, Portugal
[3] Instituto de Investigação e Inovação em Saúde (I3S), Universidade do Porto, Porto, Portugal

* Correspondence: marta.oliveira@graq.isep.ipp.pt

https://doi.org/10.48797/sl.2023.34

Abstract

Background: Occupational exposure as a firefighter has been recently classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) [1]. Polycyclic aromatic hydrocarbons (PAH) are one of the main fire-related pollutants [3], and their presence in the breathable air of firefighters was already demonstrated [4]. However, the toxicity mechanisms involved in such exposures have not yet been evaluated in human cell lines. **Objective:** The present work aimed at quantifying the PAH levels of breathable air collected in polyurethane foams (PUF) of non-exposed firefighters (control group) *vs* firefighters exposed during controlled forest fires, as well as assessing the *in vitro* toxicity of the collected PUF extracts in human alveolar (A549) and bronchial (Calu-3) epithelial cell lines. **Methods:** Firefighters used a pre-cleaned PUF foam on the breathing air zone during regular work shifts at the fire station (control group) or during three distinct controlled fire events (November 2021-February 2022). Samples were extracted by microwave-assisted extraction and analyzed by liquid chromatography with a diode array and fluorescence detectors. The PUF extracts were analyzed *in vitro* by exposing them to A549 and Calu-3 cell lines for 24h. A 3-[4,5-dimethylthiazol-2-yl]-2,5diphenyl-tetrazolium-bromide (MTT) assay was performed to assess the cell viability. **Results:** The PAH levels determined in samples from controlled fire events were 1.9 to 23.2x higher than the control group and, the levels of carcinogenic PAH were 1.9 to 15.2x higher. Most of the PUF samples from non-exposed firefighters (control group) induced a significant viability decrease (<70%) for both cell lines. Although for controlled forest fire events, an accentuated decrease in A549 and Calu-3 cellular viability (similar to the positive control in most cases) was observed. **Conclusions:** The present results demonstrated that fire- or non-fire-related occupational activities may potentially contribute to the pulmonary health burden of firefighters.

Keywords: firefighters' exposure; PAH; pulmonary cell lines; cell viability

Acknowledgments

This work was supported by projects from the Portuguese Foundation for Science and Technology (PCIF/SSO/0090/2019 and 2022.05381.PTDC). M. J. Bessa is thankful to the postdoc grant from project 2022.05381.PTDC. M. Oliveira and F. Rodrigues are thankful for their scientific contracts (CEECIND/03666/2017 and CEECIND/01886/2020, respectively).

References

1. Demers, P. A.; DeMarini, D. M.; Fent, K. W.; Glass, D. C.; Hansen, J.; Adetona, O.; Andersen, M. H. G.; Freeman, L. E. B.; Caban-Martinez, A. J.; Daniels, R. D.; Driscoll, T. R.; Goodrich, J. M.; Graber, J. M.; Kirkham, T. L.; Kjaerheim, K.; Kriebel, D.; Long, A. S.; Main, L. C.; Oliveira, M.; Peters, S.; Teras, L. R.; Watkins, E. R.; Burgess, J. L.; Stec, A. A.; White, P. A.; DeBono, N. L.; Benbrahim-Tallaa, L.; de Conti, A.; El Ghissassi, F.; Grosse, Y.; Stayner, L. T.; Suonio, E.; Viegas, S.; Wedekind, R.; Boucheron, P.; Hosseini, B.; Kim, J.; Zahed, H.; Mattock, H.; Madia, F.; Schubauer-Berigan, M. K., Carcinogenicity of occupational exposure as a firefighter. *Lancet Oncol* ***2022****, 23, (8), 985 - 986.*
2. International Agency for Research on Cancer, Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. In IARC Monogr Eval Carcinog Risks Hum: Lyon, France, **2010**; Vol. 92.
3. Barros, B.; Oliveira, M.; Morais, S., Firefighters' occupational exposure: Contribution from biomarkers of effect to assess health risks. *Environ Int* **2021,** 156, 106704-10672
4. Kirk, K. M.; Logan, M. B., Firefighting Instructors' Exposures to Polycyclic Aromatic Hydrocarbons During Live Fire Training Scenarios. *J Occup. Environmental Hyg* **2015,** 12, (4), 227-234.

Poster 36

MDA effects on morphophysiology and reproduction of *Daphnia magna* – preliminary data

A. M. Morão [1,*], A. R. Carvalho [1], C. Couto [1,2], J. S. Carrola [3,4], P. Costa [5,6], B. Castro [5,6], M. E. Tiritan [1,7,8] and C. Ribeiro [1]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[2] LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, CITAB, Vila Real, Portugal
[4] Inov4Agro – Institute for Innovation, Capacity Building and Sustainability of Agri-food Production
[5] CBMA - Centre of Molecular and Environmental Biology / ARNET - Aquatic Research Network, University of Minho, Portugal
[6] IB-S - Institute of Science and Innovation for Bio-Sustainability, University of Minho, Portugal
[7] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal
[8] Interdisciplinary Center of Marine and Environmental Research (CIIMAR), University of Porto, Edifício do Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal

* Correspondence: a28150@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.37

Abstract

Background: Psychoactive substances and their metabolites are considered emergent contaminants, raising environmental concerns due to the continuous input into aquatic ecosystems [1]. MDA (3,4-methylenedioxyamphetamine) is a pharmacologically active substance that represents the major metabolite of 3,4-methylenedioxymethamphetamine (MDMA); MDA may be present in MDMA preparations or occur as an illegal psychoactive substance [2]. Considering the possible implementation of MDMA-assisted psychotherapy along with the increased recreational interest in MDA, the presence of MDA in aquatic ecosystems is expected to increase as well as its effects on non-target organisms, including invertebrates [1,3]. Since the ecotoxicological impact of MDA remains unknown, studying its toxic effects on an environmentally relevant organism is most important. **Objective:** This work aimed to assess the effects of possible MDA-induced toxicity on *Daphnia magna* through the evaluation of morphophysiological and reproductive endpoints. **Methods:** Neonates (<24 hours) were exposed to three concentrations of MDA (0.1, 1 and 10 mg/L) for 9 days. Exposures and control were performed with 5 replicates with 20 organisms each. On days 3 and 9, morphophysiological endpoints (body size, heart size and area, and heart rate) and reproductive endpoints (number of ovigerous daphnia and fertility) were determined. In parallel, a standard 21-d reproduction assay was conducted (MDA concentrations: 0, 0.10, 0.18, 0.32, 0.56, 1.00, 1.79 mg/L). **Results:** Morphophysiological endpoints increased with MDA concentration on the third and ninth day of exposure. An increase in the number of ovigerous daphnia and fecundity was found at 10 mg/L of MDA. In the 21-d assay, MDA caused a significant reduction in fecundity, body size and rate of increase from 0.18 mg/L upwards. **Conclusions:** Data are suggestive of some adverse influence of MDA on the analyzed endpoints. Nevertheless, other toxicity biomarkers should be evaluated to obtain insight on a mechanistic explanation for the observed effects.

Keywords: MDA; ecotoxicity; *Daphnia magna*; morphophysiology; reproduction

Acknowledgments

This work is financially supported by national funds through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX) and supported UIDB/04033/2020 (CITAB).

References

1. Fontes MK, Maranho LA, Pereira CDS. Review on the occurrence and biological effects of illicit drugs in aquatic ecosystems. *Environ Sci Pollut Res Int*, 2020, 27: 30998-31034.
2. Baggott MJ, Garrison KJ, Coyle JR, Galloway GP, Barnes AJ, Huestis MA, Mendelson JE. Effects of the Psychedelic Amphetamine MDA (3,4-Methylenedioxyamphetamine) in Healthy Volunteers. *J Psychoactive Drugs*, 2019, 51: 108-117.
3. Kolaczynska KE, Ducret P, Trachsel D, Hoener MC, Liechti ME, Luethi D. Pharmacological characterization of 3,4-methylenedioxyamphetamine (MDA) analogs and two amphetamine-based compounds: N,α-DEPEA and DPIA. *Eur Neuropsychopharmacol*, 2022, 59: 9-22.

Poster 37

Changes on swimming behavior induced by 3,4-methylenedioxypyrovalerone (MDPV) enantiomers on *Daphnia magna*

A. Pérez-Pereira [1,2,*], A. Carvalho [1], B. Castro [3,4], J. S. Carrola [2], C. Fernandes [5,6], M. Tiritan [1,5,6] and C. Ribeiro [1,5]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, 4585-116 Gandra, Portugal
[2] Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, CITAB, Vila Real, Portugal
[3] CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal
[4] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Braga, Portugal
[5] Interdisciplinary Center of Marine and Environmental Research (CIIMAR), University of Porto, Edifício do Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal
[6] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

* Correspondence: ariana.pereira@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.36

Abstract

Background: Synthetic cathinones (SC) are a group of novel illicit psychotropic substances that have been found in the aquatic environment at low concentrations [1] and nonetheless they can impact aquatic invertebrates [2]. SC can pose unwanted diverse adverse effects such as behavioral toxicity to non-target organisms [3]. The 3,4-methylenedioxypyrovalerone (MDPV) is a chiral SC with psychotropic properties similar to methamphetamine and is traded as "bath salts" [4]. MDPV may be found in distinct forms, racemate or its enantiomers, that may exhibit different biological activities [2, 5]. Nevertheless, MDPV enantiotoxicity continued to be ignored as well as its impact on freshwater aquatic organisms, including invertebrates. **Objective:** This work aimed to assess the potential adverse effects of (*R*)-MDPV and (*S*)-MDPV on the swimming behavior of *Daphnia magna*. **Methods:** For that, neonates (< 24 hours) were exposed to 0.1 and 1.0 µg L^{-1} of both MDPV enantiomers for 5 days (5 replicates with 20 organisms each). After the end of exposure, 6 organisms of each replicate were randomly collected, placed into a 6-well plate (with ≈ 5 mL of the respective exposure medium) and video recorded for 1 minute. Parameters such as swimming speed, total distance travelled, and active time were evaluated using TheRealFishTracker program. **Results:** A significant increase in swimming speed was observed for the organisms exposed only to (*S*)-MDPV. On the contrary, an increase in active time was found in the organisms exposed to (*R*)-MDPV. No changes were detected for the total distance travelled for both enantiomers. **Conclusions:** This study showed that MDPV can interfere with the swimming behavior pattern of daphnia and that effects are enantioselective. However, for a better understanding of the enantioselective toxicity of MDPV on the fitness of daphnia, other parameters should be included (i.e., morphophysiological, reproductive and biochemical).

Keywords: chiral psychotropic drugs; MDPV; enantiotoxicity; aquatic pollution; emergent contaminants

Acknowledgments

Ariana Pérez-Pereira acknowledges the PhD grant BD/CBAS/CESPU/04/2022 (from February 1, 2022 to January 31, 2023), and FCT PhD grant 2022.09843.BD (since February 1, 2023). This work is financed by Portuguese national funds through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020. Partially supported by FCT - Foundation for Science and Technology through the projects UIDB/04033/2020 (CITAB); UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry - CIIMAR).

References

1. Castiglioni S.; Salgueiro-González N.; Bijlsma L.; Celma A.; Gracia-Lor E.; Beldean-Galea M.; Mackuľak T.; Emke E.; Heath E.; Kasprzyk-Hordern B.; Petkovic A.; Poretti F.; Rangelov J.; Santos M.; Srema˘cki M.; Styszko K.; Hernández F.; Zuccato E. New psychoactive substances in several European populations assessed by wastewater-based epidemiology. *Water Res* **2021**, *195*, 116983.
2. Ribeiro A.; Maia A.; Ribeiro C.; Tiritan M. Analysis of chiral drugs in environmental matrices: Current knowledge and trends in environmental, biodegradation and forensic fields. *TrAC Trend Anal Chem* **2020**, *124*, 115783.
3. Kuropka P.; Zawadzki M.; Szpot P. A review of synthetic cathinones emerging in recent years (2019–2022). *Forensic Toxicol* **2022**, *41*(1), 25-46.
4. Soares J.; Costa V.; Bastos M.; Carvalho F.; Capela J. An updated review on synthetic cathinones. *Arch Toxicol* **2021**, *95*(9), 2895-2940.
5. Pérez-Pereira A.; Ribeiro C.; Teles F.; Gonçalves R.; Goncalves V.; Pereira J.; Carrola J.; Pires C.; Tiritan M. Ketamine and norketamine: enantioresolution and enantioselective aquatic ecotoxicity studies. *Environ Toxicol Chem* **2021**, *41*(3), 569-579.

Poster 38

Butylone effects on the behaviour of zebrafish (*Danio rerio*) larvae

O. Ribeiro [1,2], L. Félix [1,3], C. Ribeiro [2,4] and J. S. Carrola [1,3,*]

[1] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[3] Inov4Agro, Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, Vila Real, Portugal
[4] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal

* Correspondence: joao@utad.pt

https://doi.org/10.48797/sl.2023.28

Abstract

Background: New psychoactive substances (NPS) represent an increasing human health and environmental concern. Despite the efforts to control their production and consumption, new molecules are identified every year. Consequently, their presence in water bodies has been reported raising concern due to potential negative impacts on non-target species such as fish [1], including the synthetic cathinone butylone (BTL) [2]. No information was found regarding its effects on exposed fish, like *Danio rerio*. This vertebrate is a valorous animal model in numerous areas of toxicology research, particularly to investigate the potential adverse effects of NPS during early-life stages [3]. **Objective:** This study aims to assess the potential effects of BTL on zebrafish larvae behaviour. **Methods:** *D. rerio* embryos with approximately 3 hours post-fertilization (hpf) were exposed until 96 hpf in triplicate to different concentrations of BTL (0.01, 0.1, 1, 10 and 100 µg/L). At 120 hpf, behavioural parameters were assessed in larvae: speed, distance to the centre of the well, absolute turn angle, total distance moved and active time. **Results:** Overall, an increase in speed, absolute turn angle and total distance moved was observed for the organisms exposed to the higher concentrations1 and 10 µg/L compared with the control group; however, no statistical differences were observed. **Conclusions:** No changes were found on swimming behaviour of the larvae exposed at the selected BTL concentrations. Furthermore, these concentrations are much higher than the environmental relevant concentrations (0.01 µg/L) usually found. These findings suggest that the exposure to environmental relevant concentrations of BTL during early life stages should not affect early life stages of wild fish. Further research is necessary to study the effects of BTL on fish under chronic exposure to estimate the potential impacts on wild fish populations and improve risk assessment.

Keywords: new psychoactive substances; butylone; zebrafish larvae; behaviour

Acknowledgments

This work was financially supported by Portuguese National Funds by Foundation for Science and Technology (FCT), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX) and UIDB/04033/2020 (CITAB/Inov4Agro). Ondina Ribeiro thanks the FCT for the PhD Grant 2022.12242.BD.

References

1. Bade, R., et al., Quantification of new psychoactive substances in Australian wastewater utilising direct injection liquid chromatography coupled to tandem mass spectrometry. *Talanta* **2023**, *251*: p. 123767.
2. Spálovská, D., et al., Structure determination of butylone as a new psychoactive substance using chiroptical and vibrational spectroscopies. *Chirality* **2018**, *30*(5): p. 548-559.
3. Ribeiro, O., et al., Effects of acute metaphedrone exposure on the development, behaviour, and DNA integrity of zebrafish (Danio rerio). *Environ. Sci. Pollut. Res*. **2023**, p. 1-10.

Poster 39

Butylone enantioseparation and ecotoxicity evaluation on *Daphnia magna*

A. R. Carvalho [1,2,*], A. M. Morão [1], V. M. F. Gonçalves [1,3], M. E. Tiritan [1,4,5], A. R. Lado [6,7], J. S. Carrola [8,9], M. M. Amorim [2] and C. Ribeiro [1,*]

[1] TOXRUN-Toxicology Research Unit, University Institute of Health Sciences, IUCS-CESPU, CRL, 4585-116 Gandra, Portugal
[2] CISA, ESS, Polytechnic Institute of Porto, Rua Dr. António Bernardino de Almeida, 400, 4200-072 Porto, Portugal
[3] UNIPRO-Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal
[4] Interdisciplinary Center of Marine and Environmental Research (CIIMAR), University of Porto,4450-208 Matosinhos, Portugal
[5] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[6] LSRE-LCM - Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[7] ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[8] Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, CITAB, Vila Real, Portugal
[9] Inov4Agro – Institute for Innovation, Capacity Building and Sustainability of Agri-food Production

* Correspondence: anartcarvalho@gmail.com; claudia.ribeiro@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.13

Abstract

Background: The synthetic drug butylone (2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one, BTL) is a chiral cathinone consumed in the form of racemate [1]. After consumption, BTL is metabolized and excreted along with its metabolites, and its residues are carried by sewerage systems to wastewater treatment plants (WWTPs) [2]. Both human metabolism and biodegradation in WWTPs may be enantioselective causing a change in its enantiomeric fraction (EF). However, enantiomers may exhibit different biological activities including toxicity on non-target aquatic organisms [3]. **Objective:** This study aimed to separate both enantiomers and assess the sub-chronic effects on *Daphnia magna* focusing on morphophysiological and reproductive parameters. **Methods:** The enantiomers were separated by liquid chromatography using a homemade semipreparative chiral column (APS-Nucleosil coated with a 3,5-dimethylphenylcarbamate of amylose). Daphnia (with less than 24 h) were exposed for 9 days to concentrations of 0.1, 1, or 10 µg/L, with a total of 5 replicates per concentration and a control. **Results:** Morphophysiological alterations were observed, except in the heart area. A tendency to the increase of body size, heart size and mortality were observed for the higher concentrations (1 and 10 µ/L). The daphniids with eggs tended to decrease. Analysis of other endpoints (ongoing) are required to draw accurate conclusions. **Conclusions:** The present study demonstrates that exposure to BTL may cause effects on mortality and morphology of *D. magna*. The ongoing studies will bring new knowledge on BTL adverse effects and the possible enantioselective toxicity effects on this non-target aquatic organism.

Keywords: ecotoxicity; psychoactive drugs; *Daphnia magna*; enantioseparation

Acknowledgments

This work is financially supported by national funds through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020. ARLR acknowledges the support from LA/P/0045/2020 (ALiCE), UIDB/04033/2020 (CITAB/Inov4Agro), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM), funded by national funds through FCT/MCTES (PIDDAC), and FCT funding under the Scientific Employment Stimulus - Individual Call (2022.00184.CEECIND).

References

1. Roque Bravo, R.; Carmo, H.; Valente, M.J.; Silva, J.P.; Carvalho, F.; Bastos, M.L.; Dias da Silva, D. From street to lab: in vitro hepatotoxicity of buphedrone, butylone and 3,4-DMMC. *Arch Toxicol* **2021**, *95*, 1443-1462.
2. Wang, Z.; Tang, B.; Wang, K.; Hao, Y.; Yang, F. Accumulation and risk prioritization of psychoactive substances in the critically endangered Yangtze finless porpoise. *J. Hazard. Mater.* **2023**, *442*, 130002.
3. Langa, I.; Tiritan, M.E.; Silva, D.; Ribeiro, C. Gas Chromatography Multiresidue Method for Enantiomeric Fraction Determination of Psychoactive Substances in Effluents and River Surface Waters. *Chemosensors* **2021**, *9*, 224.

Poster 40

Effects of methylenedioxypyrovalerone (MDPV) in avoidance behaviour and reproduction on the earthworm *Eisenia fetida*

O. Ribeiro [1,2,3,4], J. Ferreira [1], J. R. Sousa [1,2,5], T. Natal-da-Luz [4], C. Ribeiro [3,6] and J. S. Carrola [1,2,5,*]

[1] University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), UTAD, Vila Real, Portugal
[3] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[4] University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Coimbra, Portugal
[5] Inov4Agro, Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, Vila Real, Portugal
[6] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal

* Correspondence: joao@utad.pt

https://doi.org/10.48797/sl.2023.14

Abstract

Background: The excessive use of psychoactive substances (PAS) is a global problem that directly and indirectly affects human, animal health and environment [1], related with "one health" concept. These substances and their metabolites enter in the wastewater through human excretion and consequently into water systems. The sewage sludge produced in wastewater treatment plants can be used as amendment in agricultural soils leading to their contamination [2]. Methylenedioxypyrovalerone (MDPV) is a recent PAS known as "bath salts" [3] found in recreational settings. Due to the increase of PAS in soil, it is important to evaluate its toxic effects on soil organisms, like the earthworm *Eisenia fetida*, considered soil health bioindicators and an ecologically significant model species [4]. **Objective:** The study aim was to assess the impact of MDPV on the behaviour and reproduction of *E. fetida*. **Methods:** Following the procedures described in the ISO 17512-1, dual chambre Avoidance Tests were conducted for 48 hours, using adults *E. fetida* that were exposed to combinations of uncontaminated soil and contaminated soils with different MDPV concentrations (25, 250, and 2500 µg/kg). Additionally, a Reproduction Test was carried out using the same earthworm species and MDPV concentrations, over 56 days, following the procedures described in ISO 11268-2. In both tests, treatments were conducted in triplicate. **Results:** No statistically significant effects were observed on either avoidance behaviour or reproduction assays of adult earthworms exposed to the different concentrations of MDPV. **Conclusions:** Our data suggest that the MDPV concentrations tested do not induce adverse effects on avoidance behaviour and reproduction at concentrations lower or equal to 2500 µg/kg. Thus, the results suggest that no negative effects will be observed on earthworm populations exposed to MDPV. Nevertheless, it can be useful to assess MDPV effects on other key fauna organisms representing other taxonomic groups, like mites and collembolans.

Keywords: psychoactive substances; MDPV; *Eisenia fetida*; avoidance behaviour; reproduction test

Acknowledgments

This work was financially supported by Portuguese National Funds by Foundation for Science and Technology (FCT), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX) and UIDB/04033/2020 (CITAB/Inov4Agro). Ondina Ribeiro thanks the FCT for the PhD Grant 2022.12242.BD.

References

1. Cunha, D.L., F.G. de Araujo, and M. Marques, Psychoactive drugs: occurrence in aquatic environment, analytical methods, and ecotoxicity—a review. *Environ. Sci. Pollut. Res.* **2017**, *24*(31): p. 24076-24091.
2. Govindarasu, P., *Illicit drugs: environmental occurrence, fate and toxicity.* Newcastle. University of Newcastle, Faculty of Science & Information Technology, Global Centre for Environmental Remediation, University of Newcastle Research Higher Degree Thesis, 2016.
3. Baumann, M.H., et al., *Powerful cocaine-like actions of 3, 4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. Neuropsychopharmacology* **2013**, *38*(4): p. 552-562.
4. Chen, X., et al., Oxidative stress responses and insights into the sensitivity of the earthworms Metaphire guillelmi and Eisenia fetida to soil cadmium. *Sci. Total Environ.* **2017**, *574:* p. 300-306.

Poster 41

Impact of environmental caffeine contamination in zebrafish: ecotoxicological approach

I. Pinto [1,2,3,4,*], B. S. Diogo [1,2,3], S. C. Antunes [2,3] and S. Rodrigues [2,3]

[1] ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[2] CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal.
[3] FCUP, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre S/N. 4169-007 Porto, Portugal
[4] UMIB-ICBAS, Unidade Multidisciplinar de Investigação Biomédica - Instituto Ciências Abel Salazar da Universidade do Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

* Correspondence: ivofilipemp@fc.up.pt

https://doi.org/10.48797/sl.2023.38

Abstract

Background: Caffeine (CAF) is considered a suitable indicator of anthropogenic contamination of aquatic environments, and several pharmacological and biological effects have been observed in target and non-target organisms [1,2]. **Objective:** Assess the chronic effects of environmental concentrations of CAF in several biomarker responses in *Danio rerio*. **Methods:** After chronic exposure to CAF (0.16, 0.42, 1.09, 2.84, 7.40, 19.23, and 50 µg/L) on *Danio rerio,* several biomarkers of antioxidant defense (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRed) activities, and glutathione (GSH) content), biotransformation (glutathione *S*-transferase activity (GSTs)), lipid peroxidation (thiobarbituric acid reactive substances (TBARS) levels), energetic reserves (glycogen (GLY), lipids (LIP), protein (PROT) contents, and lactate dehydrogenase (LDH) activity), and neurotransmission (acetylcholinesterase (AChE) activity) were evaluated. A multi-biomarker approach known as Integrated Biomarker Response (IBR) was also employed to perceive the most sensitive and responsive biomarker, after CAF exposure. **Results:** Exposure to CAF disrupts several metabolic pathways in *D. rerio, such* as changes in antioxidant defenses, specifically in the activities of SOD, GRed, and GSH content, which may have led to lipid peroxidation. LDH activity decreased at all tested concentrations, while AChE activity was only affected at the highest concentrations (19.23 and 50 µg/L). IBR revealed that the highest stress occurred at a concentration of 50 µg/L and showed clearly that biomarker responses were responsive and coherent to demonstrate the stress caused independently to CAF concentration in fish. **Conclusions:** These results highlight the urgent need to minimize the increasing load of CAF on the aquatic ecosystems, considering the adverse impacts of CAF pollution in the aquatic environment. These results show that the implementation of scientific programs and monitoring projects are imperative to classify the CAF as a high-priority environmentally hazardous emerging pollutant.

Keywords: *Danio rerio*; chronic exposure; physiologically-based ecotoxicity; biochemical biomarkers

Acknowledgments

This research was funded by Foundation for Science and Technology, and by the Strategic Program UIDB/04423/2020 and UIDP/04423/2020. Sara Antunes and Sara Rodrigues are hired through the Regulamento do Emprego Científico e Tecnológico – RJEC from the FCT program (CEECIND/01756/2017 and 2020.00464.CEECIND, respectively). Bárbara Diogo and Ivo Pinto were supported by FCT Ph.D. grants (2022.10505.BD and 2022.l0194.BD respectively).

References

1. Abdelkader, T.S., Chang, S.N., Kim, T.H., Song, J., Kim, D.S., Park, J.H., 2013. Exposure time to caffeine affects heartbeat and cell damage-related gene expression of zebrafish Danio rerio embryos at early developmental stages. *J Appl Toxicol* 33, 1277–1283.
2. Ferreira, A.P., de Lourdes, C., da Cunha, N., 2005. Anthropic pollution in aquatic environment: Development of a caffeine indicator. *Int J Environ Health Res* 15, 303-311.

Poster 42

Enantiomeric profiling and drug consumption estimation

I. Langa [1,*], A. R. L. Ribeiro [2,3], N. Ratola [3,4], V. M. F. Gonçalves [1,5], M. E. Tiritan [1,6,7] and C. Ribeiro [1]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[2] Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
[3] ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
[4] LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[5] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal
[6] Interdisciplinary Center for marine and Environmental Research (CIIMAR), Port of Leixões Cruise Terminal, Av. General Norton de Matos, s/n, Matosinhos, Portugal
[7] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

* Correspondence: ivan.langa@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.39

Abstract

Background: Recent trends in new psychoactive substances (NPS) abuse have raised a serious public health issue that affects all communities. NPS comprise a wide range of substances, such as synthetic cathinones, synthetic cannabinoids, opioids, and benzodiazepines [1, 2]. These substances end up in the environment as excretion products or by direct disposal [3]. Wastewater-based epidemiology (WBE) combined with the evaluation of enantiomeric fractions (EF) is used for complementing the drug monitoring methods traditionally used to estimate drug consumption. However, the adsorption of these substances to suspended particulate matters (SPM) has been neglected, leading to a potential underestimation of the consumption patterns [3, 4]. **Objective:** This study was aimed to better understand and to get knowledge on: (i) the enantiomeric profiling and consumption estimation of amphetamine-type substances (amphetamine (AMP), methamphetamine, 3,4-methylenedioxymethamphetamine) and synthetic cathinones (buphedrone, butylone, 3,4-dimethylmethcathinone and 3-methylmethcathinone); (ii) the behaviour and distribution of NPS in SPM. **Methods:** In this study, 24-h composite raw wastewaters were collected from a wastewater treatment plant (WWTP) located in the north of Portugal. After the extraction, the SPM and influent extracts were analyzed using an indirect analytical method. Briefly, samples were subjected to chiral derivatization using (*R*)-(−)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride, leading to the formation of diastereomers that were further analyzed by gas chromatography coupled to mass spectrometry (GC–MS), as described elsewhere [3]. **Results:** Both enantiomers of AMP, MDMA and 3,4-DMMC and (*S*)-MAMP and the first eluted enantiomer of BPD and 3-MMC were detected. The selected NPS were found at concentrations between LOQ and 0.4 ng mg^{-1} and <LOQ and 315,82 ng L^{-1} in SPM and influents, respectively. Regarding the consumption estimation, AMP showed the highest values (<166,05 mg d^{-1} 1000 inh^{-1}). **Conclusions:** The method allowed the characterization of the adsorption as well as the assessment of consumption patterns, occurrence, and the EF of the target chiral NPS.

Keywords: synthetic cathinones; suspended particulate matter; amphetamine type substances; wastewater treatment plants; chirality

Acknowledgments

This research was funded by FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020. ARR and NR acknowledge the financial support from LA/P/0045/2020 (ALiCE), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM), and UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), and Project "HealthyWaters" (NORTE-01-0145-FEDER-000069), co-financed by NORTE 2020, through Portugal 2020 and FEDER; ARR acknowledges FCT funding under the Scientific Employment Stimulus - Individual Call (2022.00184.CEECIND).

References

1. EMCDDA, EDR 2022: Trends and Developments,POEU, **2022**.
2. UNODC, WDR 2022, Booklet 3, Drug Market Trends: Opioids, Cannabis (*UNP, 2022*), **2022**.
3. Langa, I.M.; Tiritan, M.E.; Silva, D.; Ribeiro, C., Gas Chromatography Multiresidue Method for Enantiomeric Fraction Determination of Psychoactive Substances in Effluents and River Surface Waters. *Chemosensors*, **2021**. **9**(8): p. 224.
4. Langa, I.M.; Gonçalves, R.; Tiritan, M. E.; Ribeiro, C. Wastewater analysis of psychoactive drugs: Non-enantioselective vs enantioselective methods for estimation of consumption. *Forensic Sci Int*, **2021**. 325: p. 110873.

Poster 43

The ecotoxicity evaluation of Gens ponds: are the pH values a confounding factor?

R. Pereira [1,*], B. S. Diogo [1,2,3], I. Rodrigues [4] and S. C. Antunes [1,3]

[1] FCUP, Department of Biology, Faculty of Sciences University of Porto, Rua do Campo Alegre S/N, 4169-007, Porto, Portugal, Country
[2] ICBAS, Abel Salazar Institute of Biomedical Sciences University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[3] CIIMAR, Interdisciplinary Center of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N, 4450-208, Matosinhos, Portugal
[4] Gondomar Town Hall, Praça Manuel Guedes, 4420-193, Gondomar, Portugal

* Correspondence: up201809620@edu.fc.up.pt

https://doi.org/10.48797/sl.2023.40

Abstract

Background: Portuguese polymetallic mining activities were important for local development of economy and ceased in 1970s. However, these activities leave amounts of tailings susceptible to erosion and chemical weathering, representing a potential risk to the environment, due to a high concentration of heavy metals and acidic mine drainages [1,2]. Gens ponds arose due to open pit mining in a gold-antimony mine in Gondomar (north of Portugal). **Objective:** Evaluate the ecotoxicological effect of natural water samples from these ponds in *Lemna minor (*growth inhibition) and *Daphnia magna* (acute immobilization, survival, and feeding rate inhibition assays) along seasons from one year. **Methods:** The assays were performed according to standard guidelines using the natural water samples from each pond (P1, P2, and P3) and the same samples with pH adjustment (BP1, BP2, and BP3) according to the optimal value for the organisms tested. **Results:** *L. minor* showed a significant decrease in fronds number in all samples. However, after pH adjustment, the number of fronds increase significantly in summer samples (BP1 and BP2). *D. magna* exposed to P1 and P2 showed 100% mortality in less than 24 h, while P3 only after 48h was recorded mortality. Overall, BP1, BP2, and BP3 do not affect *D. magna* survival, however, a significant decrease in the feeding rate was observed, in BP1 and BP2 in summer samples. Nevertheless, the winter samples showed an increase in feeding rate after exposure to BP1, BP2, and BP3. **Conclusions:** The results emphasize the importance of conducting ecotoxicological studies in acidic mine drainage, as Gens Ponds, to assess the effects on the ecosystem health. Further studies will be necessary to evaluate the toxicity of these ponds, considering other physical and chemical characteristics (e.g., metals and nutrients), as well as other model organisms and endpoints from different levels of biological organization.

Keywords: acidic mine drainage; ecotoxicology; *Daphnia magna*; *Lemna minor*

Acknowledgments

This work was supported by the strategic programs UIDB/04423/2020 and UIDP/04423/2020. Sara Antunes are hired through the Regulamento do Emprego Científico e Tecnológico—RJEC from the Portuguese Foundation for Science and Technology (FCT) program (CEECIND/01756/2017). Bárbara S. Diogo was supported by a FCT PhD grant (ref. 202210505.BD).

References

1. Antunes, I.M.H.R.; Albuquerque, M.T.D. Using Indicator Kriging for the Evaluation of Arsenic Potential Contamination in an Abandoned Mining Area (Portugal). *Science of the Total Environment* **2013**, *442*, 545–552.
2. Pereira, R.; Ribeiro, R.; Gonçalves, F. Plan for an Integrated Human and Environmental Risk Assessment in the S. Domingos Mine Area (Portugal). *Human and Ecological Risk Assessment* **2004**, *10*, 543–578.

Poster 44

Ecotoxicological effects of elutriates from coal mining waste in *Lactuca sativa* seed germination

A. Rajnauth [1,*], B. S. Diogo [2,3], J. E. Marques [1], D. Flores [1] and S. C. Antunes [3]

[1] Departamento de Geociências, Ambiente e Ordenamento do Território e Instituto de Ciências da Terra, Faculdade de Ciências da Universidade do Porto, 4169-007 Portugal
[2] ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[3] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Departamento de Biologia da Faculdade de Ciências da Universidade do Porto, Porto, Portugal

* Correspondence: up201912339@fc.up.pt

https://doi.org/10.48797/sl.2023.41

Abstract

Background: Fojo mine (Castelo de Paiva, Douro Carboniferous Basin) was used for anthracite exploitation until 1994, resulting in the production of coal waste piles without any environmental management [1,2]. In October 2017, a wildfire triggered the ignition of some of these waste piles. The extinguishing process of the fire included the remobilization of the coal mining residues using water mixed with a cooling accelerator agent. **Objective:** To assess the ecotoxicological effects of soil elutriates from the Fojo coal mine waste in seed germination assay with *Lactuca sativa*. **Methods:** 25 soil samples were taken: unburned coal waste (ENA); burned coal waste (EA); burned coal waste cover (EAC); mixed material (EE) resulting from the extinguishing process; uphill from the waste pile (B); downhill from the waste pile (SJ). The assay was performed with elutriates from each soil sample and four replicates were prepared per elutriate with 10 seeds. The percentage of germination, fresh biomass, and total size were measured at the end of 14 days. **Results:** The percentage of seed germination, leaf, and root growth were differently affected revealing high heterogeneity of soil characteristics. The highest seed germination was in EE soil samples (>80%), and the lowest values were in different heterogenic zones (ENA3, ENA5, EAC10, and SJ5). The lowest total size was observed in EE6 (< 4.3 cm) and ENA3 (< 5.3 cm). Leaf fresh biomass and size were higher in soil samples from B zone (1.64 g and 4.64 cm, respectively), and lower values in SJ (0.63 g and 3.34 cm). **Conclusions:** The results highlight the importance of the phytotoxicity studies with coal mining waste, since the lixiviates may affect the terrestrial ecosystems, reducing plant establishment and growth. Seed germination, and shoot and root elongation proved to be a sensitive endpoint to evaluate the phytotoxicity of coal waste.

Keywords: phytotoxicity; seed germination; *Lactuca sativa*; coal mining waste

Acknowledgments

This research was supported by SHS Project, funded by NORTE-45-2020-75- SISTEMA DE APOIO À INVESTIGAÇÃO CIENTÍFICA E TECNOLÓGICA - "PROJETOS ESTRUTURADOS DE I&D- HORIZONTE EUROPA, Ref. NORTE-01-0145-FEDER-000056 and framed within the ICT GI3 activities (UIDB/04638/2020 and UIDP/04683/2020). Sara Antunes is hired through the Regulamento do Emprego Científico e Tecnológico – RJEC from the FCT program (CEECIND/01756/2017), and Bárbara Diogo is supported by FCT Ph.D. grants (2022.10505).

References

1. Ribeiro, J.; Ferreira da Silva, E.; Flores D. Burning of coal waste piles from Douro Coalfield (Portugal): Petrological, geochemical, and mineralogical characterization. Inter. *J. Coal Geol*, **2010,** *81*, 359-372.
2. Ribeiro, J.; Suárez-Ruiz, I.; Flores, D. Coal related fires in Portugal: New occurrences and new insights on the characterization of thermally affected and non-affected coal waste piles. *Inter. Jour Coal Geol*, **2022**, 252.

Poster 45

Methylparaben as a concerning environmental pollutant compromising drinking water quality

A. R. Pereira [1,2], I. Gomes [1,2] and M. Simões [1,2,*]

[1] LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal
[2] ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

* Correspondence: mvs@fe.up.pt

https://doi.org/10.48797/sl.2023.42

Abstract

Background: Drinking water distribution systems are known to harbor biofilms, even after disinfection, which constitute a source of microorganisms that may remain in the drinking water (DW) delivered through a consumer's tap [1]. Nevertheless, the presence of parabens (an anthropogenic contaminant) in DW is another problem that may affect microbial characteristics and the susceptibility to chlorine, compromising DW disinfection [2]. **Objective:** This work is pioneer in evaluating the effects of methylparaben (MP) at concentrations found in DW (15 µg/L) on biofilm characteristics and their susceptibility to disinfection. **Methods:** Dual-species biofilms formed by bacteria isolated from DW (*Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia)* were grown for 7 days on polypropylene (PPL) in the absence and presence of 15 µg/L of MP [3]. Then, MP-exposed and non-exposed biofilms were characterized in terms of culturability, density, viability, biofilm structure (thickness) and composition (content of extracellular polysaccharides) [3]. To evaluate the effect of MP exposure on biofilm susceptibility to chlorine disinfection, MP-exposed and non-exposed dual-species biofilms were treated with free chlorine solutions at 5 and 50 mg/L for 30 min. Then, those biofilms were characterized in terms of cell culturability, density and viability. **Results:** MP exposure increased cell proliferation of dual-species biofilms formed on PPL (an increase of 36% and 63% in the number of culturable and total cells, respectively) in relation to non-exposed biofilms ($P < 0.05$). MP also altered biofilm viability, structure and composition. The thickness of MP-exposed biofilms increased by 45%, while the polysaccharides content decreased by 43%. Moreover, the results showed that MP- exposed dual-species biofilms formed on PPL were more tolerant to chlorine action than non-exposed counterparts. **Conclusions:** MP exposure induces the proliferation of biofilm cells and affects biofilm structure (thickness) and composition (polysaccharides content). MP presence in DW was found to compromise chlorination efficacy, especially in systems containing PPL.

Keywords: biofilms; drinking water; methylparaben; water disinfection; tolerance

Acknowledgments

This research was funded by LA/P/0045/2020 (ALiCE), UIDB/00511/2020, UIDP/00511/2020 (LEPABE) - FCT/MCTES (PIDDAC); by the A. R. Pereira PhD scholarship (2021.06226.BD); HealthyWaters (NORTE-01-0145-FEDER-000069)- NORTE 2020/ERDF and the I. B. Gomes contract 2022.06488.CEECIND.

References

1. Chan, S.; Pullerits, K.; Keucken, A.; Persson, K.M.; Paul, C.J. Bacterial release from pipe biofilm in a full-scale drinking water distribution system. *NPJ Biofilms Microbiomes* **2019**, *5*, 9.
2. Gomes, I.; Maillard, J.-Y.; Simões, L.C.; Simões, M. Emerging contaminants affect the microbiome of water systems—strategies for their mitigation. *NPJ Clean Water* **2020**, *3*, 39.
3. Pereira, A. R.; Gomes, I. B.; and Simões, M. Impact of parabens on drinking water bacteria and their biofilms: The role of exposure time and substrate materials. *J Environ Manage* **2023**, *332*, 117413.

Poster 46

The use of nanomaterials for water splitting process: a safe solution or a risk for the environment?

C. Ganilho [1,*], A.M. Cordeiro [1], T. Andreani [1,2,3], C. M. Pereira [2] and R. Pereira [1]

[1] GreenUPorto-Sustainable Agrifood Production Research Centre & INOV4AGRO, Department of Biology, Faculty of Sciences of University of Porto, Rua Campo Alegre s/n, 4169-007, Porto, Portugal
[2] Chemistry Research Centre (CIQUP) and Institute of Molecular Sciences (IMS), Department of Chemistry and Biochemistry, Faculty of Sciences of University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[3] Centre for Research and Technology of Agro-Environmental and Biological Sciences (CITAB-UTAD) & INOV4AGRO, Quinta de Prados, 5001-801 Vila Real, Portugal

* Correspondence: catarina.ganilho@fc.up.pt

https://doi.org/10.48797/sl.2023.43

Abstract

Background: Humanity is facing challenges in a modern world of rapidly increasing demand for energy sources. The production of clean energy systems is a major challenge, prompting an urgent need to implement new sustainable technologies that can meet global society's needs. The energy solutions rely on renewable sources, cost-efficient and environmentally friendly such as hydrogen production by water splitting [1]. Efforts have been made to explore catalysts based on transition metal compounds - tungsten oxide (W) [2,3] and recently through nanotechnology with the application of tungsten nanoparticles (WNP) [4]. The use of W compounds results in environmental exposure to this metal. Ecotoxicological studies are limited, and a detailed investigation is crucial to evaluate the effect of this metal on the environment. **Objectives:** The aim of this work is to perform a toxicological comparative study of commercial W (Alfa Aesar) and WNP oxide (Sigma-Aldrich) exposure on terrestrial species (monocotyledonous *Zea mays* and *Avena sativa*, invertebrates (*Folsomia candida* and *Eisenia fetida*) and aquatic species (*Aiivibrio fischeri*, *Raphidocelis subcapitata*, *Lemna minor*, *Daphnia magna*, and *Thamos platyrus*). **Methods:** The tests with terrestrial species were performed with natural regosol (Estarreja, Aveiro, North of Portugal - 40°45′17″ N, 8°34′9″ W) contaminated with each one of the compounds (0 to 1000 mg W kg_{soil}^{-1}), tested individually. **Results:** For aquatic species, the effects of both compounds were tested at concentrations from 0 to 200 mg W L^{-1}. The results demonstrated that W negatively affected the fresh and dry biomass of plant species and the reproductive output of *F. candida*. All aquatic species were significantly affected after exposure to W, except *D. magna*. A reduction of *R. subcapitata* and *L. minor* growth rate, an inhibition of bioluminescence of *A. fischeri* and of *T. platyrus* ability to feed were also observed. **Conclusions:** Environmental safety studies showed risks for all species exposed to W and WNP.

Keywords: sustainable technologies; nanoparticles; transition metal; environmental safety

Acknowledgments

This research was funded by "Development of Sustainable Materials or Water Splitting: An integrated study from cradle to grave" was funded by FEDER through Program NORTE 2020, NORTE-01-0145-FEDER-000076. This work was partially supported by the FCT under strategic funds awarded by Research Units, namely UIDB/00081/2020—CIQUP, LA/P/0056/2020—IMS, and UIDB/05748/2020 (GreenUPorto).

References

1. Chen, X., Yang, J., Cao, Y., Kong, L., & Huang, J. Design Principles for Tungsten Oxide Electrocatalysts for Water Splitting. *Chem Electro Chem*, **2021**, 8 (23), 4427–4440.
2. Cheng, J., & Deming, T. J. Synthesis of polypeptides by ROP of NCAs. *Peptide-Based Materials*, **2011**, *310* 1–26.
3. Kollender, J. P., Mardare, A. I., & Hassel, A. W. Localized Photoelectrochemistry on a Tungsten Oxide–Iron Oxide Thin Film Material Library. *ACS Combinatorial Science*, **2013,** *15* (12), 601–608.
4. Sengul, A. B., & Asmatulu, E. Toxicity of metal and metal oxide nanoparticles: a review. In *Environmental Chemistry Letters* 2020, 18, (5) 1659–1683.

Poster 47

Airborne fibers: passive sampling and environmental contamination

J. Lucala [1,*], J. Barbosa [1,2], J. Faria [1,2] and J. C. Prata [1,3]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences–CESPU (IUCS-CESPU), 4585-116 Gandra, PRD, Portugal
[2] UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] Food Microbiology and Technology Laboratory, Department of Aquatic Production, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal

* Correspondence: a26654@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.44

Abstract

Background: Microplastics are synthetic particles or fibers <5mm [1] released from plastic objects into the environment, contaminating indoor and outdoor air and leading to human exposure with unknown consequences [2]. Only a small number of studies with a high geographical distribution have determined the concentration of airborne microplastics, especially of fibers. **Objective:** This work aimed at conducting passive sampling of airborne fibrous microplastics in four areas to study the deposition of synthetic fibers. **Methods:** Sampling was conducted on February 15th, 2023 in the CESPU's university campus (Gandra, Portugal). Three indoor areas (Library, Auditorium, Cafeteria) and one outdoor area (Balcony) were sampled in four replicates, along with four blanks. Sampling was conducted by opening previously decontaminated glass petri dishes (30 min in 1% HNO_3, distilled water, dried at 60˚C, cleaned with air jet) for approximately 4h, starting at 12:00. Fibers were photographed in an Eclipse TE2000-U microscope, controlled by the ACT-1 software, version 2.70 (Nikon); images analyzed in ImageJ, and data analysis conducted in IBM SPSS Statistics 26, considering α=0.05. **Results:** Fibrous and non-fibrous particles were detected in petri dishes. Deposited fibers showed a significantly greater length and smaller width than non-fibrous materials (length U=2477,0, p=<0.001; width U=810.5, p<0.001). The Auditorium presented significantly greater fiber lengths, except the Cafeteria (H= 10,772, p=0.029). The number of particles was only significantly different from blanks in the Library (H=14.588, p<0.006), presenting a total 3.1 particles $hour^{-1}$ with median lengths 123 µm. **Conclusions:** Despite the decontamination procedure, blanks presented particles or artifacts which interfered with analysis. Moreover, the method requires user experience and has a wide area of analysis. The higher number of particles in the Library likely originated from foot traffic and harder to clean surfaces. Nonetheless, particles sizes found in the Library were not inhalable and do not pose a risk to health.

Keywords: fibers; microplastics; human health

Acknowledgments

This research received no external funding.

References

1. Wang, Y.; Huang, J.; Zhu, F.; & Zhou, S. Airborne Microplastics: A Review on the Occurrence, Migration and Risks to Humans. *Bull Environ Contam Toxicol* **2021**, *107*(4), 657–664.
2. Prata J. C. Airborne microplastics: Consequences to human health?. *Environ Pollut* **2018,** *234*, 115–126.

Poster 48

Metal selective pressure in the modulation of the microbial community in sediments from transitional ecosystems

E. Almeida [1,2,*], R. Torre [2,3,4], O. Godinho [1,2], M. Noyer [5], C. Cravo-Laureau [5], R. Duran [5], S. C. Antunes [1,2], J. Catita [3,6] and O. M. Lage [1,2]

[1] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 1021/1055, 4169-007 Porto, Portugal
[2] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos s/n, 4450-208 Matosinhos, Portugal
[3] Paralab, SA, R. Dr. Joaquim Manuel Costa 946 B, 4420-43, Valbom, Portugal
[4] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[5] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Bât. IBEAS, BP1155, 64013 PAU cedex, France
[6] FP-I3ID, FP-ENAS/CEBIMED, and Faculty of Health Sciences - UFP, Praça de 9 de Abril 349, 4249-004 Porto, Portugal

* Correspondence: eduardamarqalmeida@gmail.com

https://doi.org/10.48797/sl.2023.45

Abstract

Background: Microorganisms in sediments of estuarine ecosystems play key roles in the biogeochemical cycles [1]. These communities, essential for maintaining the ecosystems quality, are affected by natural and anthropogenic factors, such as metals, affecting the ecosystems health [2]. **Objectives:** This work aims to understand the effect/impact of metals on brackish sediment microbial community in a mesocosm experiment. **Methods**: Corers with sediment samples were collected from the Reserva Natural Local do Estuário do Douro. These sediments, already characterized with low levels of metals, were incubated in tanks with water collected from the same site (sediments control), and another set of sediments were incubated in higher levels of copper, lead, zinc and arsenic. The sediments in the tanks were incubated under natural conditions (light and temperature), with aeration and monitored for 30 days. **Results:** At time 0 of the experiment, the sediment bacterial and the archaeal communities were similar in both control and metal sediments samples. After 30 days, a significant decrease of alpha-diversity of the bacterial community ($F = 9.24$; $p < 0.01$) was observed for the sediments exposed to higher levels of metals which was not observed for the archaeal community. However, an increase the relative abundance of Amplicon Sequence Variant (ASV) was observed for the Archaea phyla Asgardarchaeota, Micrarchaeota, Nanoarchaeota, Thermoplasmatota, and the Bacteria phyla Bacillota, Desulfobacterota, Calditrichota, Bacteroidota, Pseudomonadota in the sediments exposed to higher levels of metals. **Conclusions:** This mesocosm experiment evidences the pressure exerted by metals in the microbial prokaryotic community of estuarine sediments, changing the dynamics of these organisms in the ecological quality of these ecosystems.

Keywords: heavy metal; Archaea; Bacteria; estuarine ecosystems; metabarcoding

Acknowledgments

This research was funded by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF), BIOMIC project, grant number SOE4/P1/F0993" and by the strategical funding from FCT UIDB/04423/2020 and UIDP/04423/2020. Sara Antunes is hired through the Regulamento do Emprego Científico e Tecnológico—RJEC from the Portuguese Foundation for Science and Technology (FCT) program (CEECIND/01756/2017). Ofélia Godinho was supported by FCT Ph.D. grant (SFRH/BD/144289/2019). Eduarda Almeida was supported by FCT Ph.D. grants (SFRH/BD/125527/2016 and COVID/BD/151721/2021).

References

1. Falkowski, P.G.; Fenchel, T.; Delong, E.F. The microbial engines that drive Earth's biogeochemical cycles. *Science* **2008**, *320*(5879):1034-9.
2. Gupta, A.; Gupta, R.; Singh, R.L. Microbes and Environment. Principles and Applications of Environmental Biotechnology for a Sustainable Future. 2016, 15:43–84.

Poster 49

Study of metal presence in the sediments of three different water bodies from the north of Portugal and its impact in Archaeal community

R. Torre [1,2,3,*], I. Pinto [2,3,4,5], O. M. Lage [3,4], M. Noyer [6], E. Almeida [3,4], C. Cravo-Laureau [6], R. Duran [6], S. C. Antunes [3,4] and J. Catita [1,7]

[1] Paralab, SA, R. Dr. Joaquim Manuel Costa 946 B, 4420-43, Valbom, Portugal
[2] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos s/n, 4450-208 Matosinhos, Portugal
[4] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 1021/1055, 4169-007 Porto, Portugal
[5] UMIB-ICBAS, Unidade Multidisciplinar de Investigação Biomédica - Instituto Ciências Abel Salazar da Universidade do Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal
[6] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM UMR 525 - Bât. IBEAS, BP1155, 64013 PAU cedex, France
[7] FP-I3ID, FP-ENAS/CEBIMED, and Faculty of Health Sciences - UFP, Praça de 9 de Abril 349, 4249-004 Porto, Portugal

* Correspondence: areginatorres@hotmail.com

https://doi.org/10.48797/sl.2023.46

Abstract

Background: Human impact on the planet's biomes has been rapidly increasing over the last decades [1]. In aquatic ecosystems, an active monitoring of the water and the sediments quality is already essential to assure its suitability for human use as well as to guarantee the health of the ecosystems [2, 3]. **Objectives:** This work focuses on the study of metals concentration in transitional water bodies (Ave estuary – 2 sites, Douro estuary – 3 sites; and Ria de Aveiro – 3 sites), and in the establishment of correlations with parameters to assess ecosystem's health based on the analysis of the less studied domain, the Archaea. **Methods:** Sediment samples were collected from the three target ecosystems. Metals concentration (Cu, Mn, Ni, Zn, Pb, Cr, Cd, As) was determined by flame atomic absorption and Archaea profiling was performed by 16S rRNA gene sequencing. The database was normalized for statistical analysis, namely for univariate (correlation matrix), and multivariate analysis (PCA, HCA). **Results:** The characterization of Archaeal community revealed the presence of 12 phyla (and several unidentified sequences). Regarding metals, Ave2 (upstream), showed high values of Cu, Mn, Zn, Cr and As, in Douro sites Cu, Mn, Zn and As were detected while in Ria de Aveiro As was the most abundant metal. Higher microbial Amplicon Sequence Variant (ASV) richness and Shannon's diversity index are related to the three sites of Ria de Aveiro, including a saltern site (≈55 PSU), which is the more extreme environment of all the sampling locations. The statistical analysis showed that in general, metals negatively affected the biodiversity of Archaeal community, with copper and chromium being the most relevant. **Conclusions:** The archaeal community in these target ecosystems is influenced by the metals present in their sediments.

Keywords: heavy metal; Archaea; aquatic ecosystems; metabarcoding

Acknowledgments

This research was funded by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF), BIOMIC project, grant number SOE4/P1/F0993" and by the strategical funding from FCT UIDB/04423/2020 and UIDP/04423/2020. Sara Antunes is hired through the Regulamento do Emprego Científico e Tecnológico—RJEC from the Portuguese Foundation for Science and Technology (FCT) program (CEECIND/01756/2017). Ivo Pinto was supported by FCT Ph.D. grants (2022.l0194.BD). Eduarda Almeida was supported by FCT Ph.D. grants (SFRH/BD/125527/2016; COVID/BD/151721/2021).

References

1. Akhtar, N.; Syakir Ishak, M.I.; Bhawani, S.A.; Umar, K. Various Natural and Anthropogenic Factors Responsible for Water Quality Degradation: A Review. *Water* **2021**, *13*, 2660.
2. Catianis, I.; Secrieru, D.; Pojar, I.; Grosu, D.; Scrieciu, A.; Pavel, A. B.; Vasiliu, D. Water Quality, Sediment Characteristics and Benthic Status of the Razim-Sinoie Lagoon System, Romania. *Open Geosci* **2018**, *10*, 12-33.
3. Rahman, M. S.; Saha, N.; Molla, A. H. Potential ecological risk assessment of heavy metal contamination in sediment and water body around Dhaka export processing zone, Bangladesh. *Environ Earth Sci* **2014**, *71*, 2293–2308.

Poster 50

Toxicological impact of microplastics on the aquatic environment and interaction with other pollutants

B. Suordem [1,2,*]

[1] FMUP – Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, IUCS-CESPU, CRL, 4585-116 Gandra, Portugal

* Correspondence: beatrizsuordem@hotmail.com

https://doi.org/10.48797/sl.2023.91

Abstract

Background: Microplastic (MP) pollution is a growing concern all over the world. MPs are derived from various petroleum compounds with a particle size of less than 5 mm [1]. The occurrence of MPs in the aquatic environment come from different sources as land water runoff, rivers and wastewater treatment plants (WWTPs) [2]. Due to their ubiquity and difficult removal, MPs can be consumed and enter trophic levels [3]. Plastics can absorb many types of toxic compounds, including organic pollutants and trace metals. Chemicals absorbed to plastic particles can enter food chains through different pathways, enhancing bioaccumulation and/or biomagnification efficiencies [4]. **Objective:** This study aims to assess the toxicological impact of MPs in the aquatic environment and their interaction with other pollutants. **Methods:** This research was based on publications available in the ScienceDirect and Scopus databases. **Results:** As MPs are detected in plankton, invertebrates and vertebrates, meaning that aquatic organisms from different hierarchies of the food chain are exposed to contamination by MPs. A study was carried out with MPs associated with zinc showed the increase of the toxicological effects. Several studies have investigated the effects of MPs on marine invertebrates. Adverse effects were also shown in earthworms, after exposure to MPs, as fibrosis, congestion and inflammatory infiltration, organ blockage, physical damage and metabolic disturbances [2]. **Conclusions:** The toxicity of MPs in aquatic organisms and their entry into trophic levels has been reported with different organisms, although according to some studies this toxicity can be altered in the presence of other contaminants.

Keywords: microplastics; aquatic environment; water pollutants

Acknowledgments

This research received no external funding.

References

1. Dris R, Imhof H, Sanchez W, Gasperi J, Galgani F, Tassin B, Laforsch C. Beyond the ocean: contamination of freshwater ecosystems with (micro-)plastic particles. *Environmental Chemistry*, **12**: 539-550, 2015.
2. Yuan Z, Nag R, Cummins E. Human health concerns regarding microplastics in the aquatic environment - From marine to food systems. *Sci Total Environ*, **823**: 153730, 2022.
3. Rogowska J, Cieszynska-Semenowicz M, Ratajczyk W, Wolska L. Micropollutants in treated wastewater. *Ambio*, **49**: 487-503, 2020.
4. Lanctôt CM, Al-Sid-Cheikh M, Catarino AI, Cresswell T, Danis B, Karapanagioti HK, Mincer T, Oberhänsli F, Swarzenski P, Tolosa I, Metian M. Application of nuclear techniques to environmental plastics research. *J Environ Radioact*, **192**: 368-375, 2018.

Poster 51

eDNA metabarcoding as a way to evaluate myxozoan presence and diversity in the sediment of a transboundary estuary

G. Ferreira [1,*], A. Machado[1,2] and S. Rocha [1,3]

[1] School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira nº 288, Porto, Portugal
[2] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[3] Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Rua Alfredo Allen nº 208, Porto, Portugal

* Correspondence: aqb.gabriel.ferreira15@gmail.com

https://doi.org/10.48797/sl.2023.71

Abstract

Background: Myxozoans are a diverse group of cnidarian endoparasites cosmopolitan in the aquatic environment, responsible for causing serious diseases in fish [1]. Traditional methods of detection and characterization of these parasites are very cumbersome and complex by nature [1-3]. Therefore, it is essential to implement simpler, non-destructive approaches to assess myxozoan presence and diversity, such as eDNA analysis [3]. Most eDNA-based parasitological assessments focus on water samples to indicate potential disease risk. However, Turner et al. [4] demonstrated that fish DNA is more concentrated in sediment than in water, which could also be true for myxozoan DNA. **Objective:** This study aimed to compare traditional methods of myxozoan detection versus a novel eDNA approach from sediment samples. **Methods:** Sediment was collected monthly from the three distinct stretches of the Minho River estuary, near Caminha (lower estuary), Boega (middle estuary), and Morraceira (upper estuary). Collected annelids were identified and microscopically surveyed for myxozoan infection. eDNA was extracted from the sediment samples and a nested PCR protocol targeting a variable region of the 18S rDNA (450–490 bp) was performed using metabarcoding primers [3]. **Results:** A total of 1,746 oligochaetes and 327 polychaetes were isolated, among which only one oligochaete collected in September from the upper estuary displayed microscopic evidence of myxozoan infection. Actinospores were identified as belonging to the sphaeractinomyxon collective group, based on morphology and 18S rDNA sequence. Conversely, eDNA metabarcoding from sediment samples revealed positive amplification throughout the sampling period, and in all three locations. Preliminary results identified amplicons as having the highest genetic similarity with myxozoan 18S rDNA sequences. **Conclusion:** This work highlights the utility of eDNA metabarcoding of sediment samples for evaluating myxozoan presence and diversity in estuarine environments, allowing the acquisition of high-fidelity results at a faster rate and superior sensitivity than those obtained via traditional methods.

Keywords: Cnidaria; 18S rDNA; Minho River; annelids; sphaeractinomyxon

Acknowledgments

This research was funded by national funds through Foundation for Science and Technology (FCT), within the scope of the project PTDC_BIA-BMA_6363_2020, and the FCT employment contract 2022.06670.CEECIND.

References

1. Okamura, B., Gruhl, A., Bartholomew, J.L. *Myxozoan Evolution, Ecology and Development*. Springer International Publishing: Switzerland, 2015; pp. 441.
2. Bell, A.S., Ranford-Cartwright, L.C. Real-time quantitative PCR in parasitology. *Trends Parasitol.* **2002**, *18,* 337–342.
3. Hartikainen, H., Bass, D. Briscoe, A.G., Knipe, H., Green, A.J., Okamura, B. Assessing myxozoan presence and diversity using environmental DNA. *Int. J. Parasitol.* **2016**, *46*, 781–792.
4. Turner, C.R. Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biol. Conserv.* **2014**, *183*, 93–102.

Poster 52

T(AHR)getting the AHR: mapping the road of a xenobiotic sensor, from disease to a therapeutic target

I. Castro-Almeida [1,2,3,*], A. Janowska [4,5], R. Saitch [4], Y. Lian [4], A. Delgado [6], J. Protze [7] and P. Moura-Alves [1,2,4]

[1] IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
[2] I3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[3] Departamento de Química, Universidade de Aveiro, Aveiro, Portugal
[4] Ludwig Institute for Cancer Research, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom
[5] Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, United Kingdom
[6] Novo Nordisk Foundation Center for Biosustainability (NNF CfB), Technical University of Denmark, Kongens Lyngby, Denmark
[7] Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany

* Correspondence: ialmeida@i3s.up.pt

https://doi.org/10.48797/sl.2023.86

Abstract

Background: The aryl hydrocarbon receptor (AHR) is a highly conserved ligand-dependent transcription factor, which recently gained recognition, beyond its role as a toxicity sensor, as a major player in different biological circumstances [1]. Our group and others have shown that AHR modulation in different scenarios, including by therapeutic drugs, impacts disease outcomes and treatment efficacy, in conditions such as cancer and bacterial infections [1-3]. For example, therapeutic drugs designed to target other molecules also bind to and modulate AHR activity [1,2]. Albeit, the extent of clinically approved drugs with AHR modulatory properties and the elicited AHR functions is largely unknown. **Objective:** Identify drugs with AHR modulatory properties. **Methods:** The AHR modulatory properties of 3178 drugs were examined using a luciferase cell reporter assay, *in silico* binding studies, and data analysis, through Ingenuity Pathway Analysis and data mining [2,4,5]. **Results:** We unbiasedly identified 228 hits as potential AHR agonists or antagonists (including known AHR ligands, validating our approach) and calculated the respective EC50s or IC50s. Next, AHR modelling studies predicted 53 agonists and 31 antagonists to bind to AHR. According to the data analysis, we classified the hits according to their roles in different pathways, diseases, and targets. We decided to initially focus on drugs with known roles in cancer or infection. An anticancer and anti-infection molecule is currently being tested for its AHR modulatory properties, and for the assessment of the AHR role(s) in its therapeutic mechanism and drug-resistance phenotypes. Further validation studies will involve *in vitro* and *in vivo* approaches (e.g. in zebrafish). **Conclusions:** In all, we aim to gain a deeper understanding of the biology of AHR in disease and its role in resistance mechanisms and identify potential repurposing drugs to target this receptor, paving the ground for future therapeutic approaches.

Keywords: aryl hydrocarbon receptor; disease; drug therapy; drug resistance

Acknowledgments

Project supported by John Fell Fund, University of Oxford; Ludwig Institute for Cancer Research – Core Award; and H2020-WIDESPREAD-2018-951921 – ImmunoHUB.

References

1. Corre, S. *et al.* Sustained activation of the Aryl hydrocarbon Receptor transcription factor promotes resistance to BRAF-inhibitors in melanoma. *Nat. Commun.* **2018**, *9*, 4775.
2. Puyskens, A. *et al.* Aryl Hydrocarbon Receptor Modulation by Tuberculosis Drugs Impairs Host Defense and Treatment Outcomes. *Cell Host Microbe* **2020**, *27*, 238-248.e7.
3. Murray, I. A., Patterson, A. D. & Perdew, G. H. Aryl hydrocarbon receptor ligands in cancer: friend and foe. *Nat. Rev. Cancer* **2014**, *14*, 801–814.
4. Moura-Alves, P. *et al.* AhR sensing of bacterial pigments regulates antibacterial defence. *Nature* **2014**, *512*, 387–392.
5. Moura-Alves, P. *et al.* Host monitoring of quorum sensing during Pseudomonas aeruginosa infection. *Science* **2019**, *366*, eaaw1629.

Poster 53

Impact of cannabidiol and delta-9-tetrahydrocannabinol in the angiogenic role of extravillous trophoblasts

P. Alves [1,2,*], C. Amaral [1,2], N. Teixeira [1,2] and G. Correia-da-Silva [1,2]

[1] UCIBIO-REQUIMTE, Department of Biological Sciences, Laboratory of Biochemistry, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[2] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313, Porto, Portugal

* Correspondence: patriciaalves.95@hotmail.com

https://doi.org/10.48797/sl.2023.87

Abstract

Background: Cannabis is the most consumed illicit drug in Europe. Alongside, the medicinal use of cannabinoids is rising. Indeed, a formulation that combines the major phytocannabinoids, cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) in a 1:1 proportion (Sativex®), is already available for the management of multiple sclerosis. Nevertheless, a lot remains unknown in relation to cannabinoid's impact in reproductive health, namely during pregnancy. Our research group already reported the negative effects of isolated CBD and THC in placental trophoblast cells, such as the induction of apoptosis and autophagy [1,2]. **Objective:** Considering that extravillous trophoblasts (EVT) participate in the remodeling of maternal vessels and produce factors that play a role in angiogenesis during placentation, our goal was to evaluate the effects of CBD and THC, isolated or in combination (1:1), in EVT's angiogenic role, using the HTR-8/SVneo cell line (ATCC, USA). **Methods:** HTR-8/SVneo cells were treated with CBD, THC and CBD plus THC (1:1), at a concentration of 2 µM. Their effects on the gene expression of the angiogenesis-related factors *VEGFA*, *PGF*, *FLT1* and *sFLT1* were assessed through RT-PCR. Tube formation assay was used to functionally evaluate HTR-8/SVneo endothelial cell-like behavior. Activation of STAT3 signaling pathway, involved in angiogenesis, was assessed by Western blot. **Results:** The mRNA levels of *PGF* were upregulated in CBD and CBD plus THC-treated cells, while *VEGFA* and *FLT1* were not affected. On the other hand, all the treatments increased the expression of the potent anti-angiogenic factor *sFLT1* and decreased tube formation. Moreover, STAT3 was activated in all treated cells. **Conclusions:** Our results demonstrate that CBD and THC, either alone or in combination, may affect placental angiogenesis, through dysregulation of relevant players and interference on the endothelial-behavior of EVT cells. Therefore, the exposure to these cannabinoids can have a harmful impact in critical phases of placental development.

Keywords: cannabinoids; extravillous trophoblasts; placenta; angiogenesis

Acknowledgments

This research was funded by Fundação para a Ciência e Tecnologia (FCT), through P. Alves PhD grant (UI/BD/151312/2021) and C. Amaral contract (SFRH/BPD/98304/2013), and by Applied Molecular Biosciences Unit – UCIBIO (UIDB/04378/2020; UIDP/04378/2020).

References

1. Alves, P.; Amaral, C.; Teixeira, N.; Correia-da-Silva, G. Synthetic cannabinoids JWH-018, JWH-122, UR-144 and the phytocannabinoid THC activate apoptosis in placental cells. *Toxicol Lett.* **2020**, *319*, 129-137.
2. Alves, P.; Amaral, C.; Teixeira, N.; Correia-da-Silva, G. Cannabidiol disrupts apoptosis, autophagy and invasion processes of placental trophoblasts. *Arch Toxicol.* **2021**, *95*, 3393-3406.

Poster 54

In silico study of (thio)xanthones-mediated P-glycoprotein activation

J. Veiga-Matos [1,2,*], D. J. V. A. dos Santos [3], E. Sousa [4,5], M. Prieto Vicente [2], F. Remião [1] and R. Silva [1]

[1] UCIBIO/REQUIMTE, Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[2] Unidad de Toxicología, Universidad de Salamanca, España; Group of Translational Research on Renal and Cardiovascular Diseases; Instituto de Investigación Biomédica de Salamanca (IBSAL); Campus Miguel de Unamuno, Edificio Departamental, 37007, Salamanca, España
[3] CBIOS – Research Center for Biosciences and Health Technologies, Universidade Lusófona, Campo Grande, 376, 1749-024, Lisboa, Portugal
[4] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[5] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal

* Correspondence: jessicamatos1996@outlook.com

https://doi.org/10.48797/sl.2023.95

Abstract

Background: P-glycoprotein (P-gp) is an efflux transporter located at the apical membrane of important barrier tissues, playing a crucial role in the detoxification of endobiotics and xenobiotics [1]. (Thio)xanthonic derivatives have been shown to be able of activating P-gp without increasing its expression, promoting an immediate increase in the amount of transported substrates. Thus, P-gp activation limits the intracellular accumulation of harmful P-gp substrates and, consequently, reduces their toxicity [2,3]. **Objective:** The aim of this study was to elucidate, *in silico*, by molecular docking analysis, the P-gp binding sites of different (thio)xanthonic derivatives previously reported as P-gp activators, and to correlate the *in silico* predictions with *in vitro* data reported in the literature. **Methods:** Molecular Operating Environment (MOE) software was used to build all the 3D-structures (with minimized energy) and Autodock Vina was used to perform the molecular docking analysis, to obtain the affinity energy between the human P-gp model [at the drug-binding pocket (DBP) and nucleotide binding domains (NBDs) 1 and 2] and P-gp activators. The best pose was visualized and the number and type of interactions of the evaluated compounds with specific P-gp residues was verified by using the BINANA software. **Results:** The molecular docking analysis revealed that most of P-gp activators preferentially bind to DBP or NBD1. Relatively to DBP, almost all P-gp activators bind to residues located in the modulators (M)-site. Furthermore, the evaluation of interactions between P-gp activators and P-gp residues indicated a pattern, since several P-gp activators shared the same hydrophobic contacts and other interactions (hydrogen-bonds and pi-pi, t-stacking and cation-pi interactions) with specific P-gp residues. **Conclusions:** The present study confirmed that (thio)xanthonic derivatives are capable of binding to P-gp, specifically at the M-site of the DBP and at the NBD1 and, therefore, these binding interactions may potentially be involved in (thio)xanthonic derivatives-mediated P-gp activation.

Keywords: computational studies; xanthones; molecular docking; Molecular Operating Environment (MOE) software; BINANA software

Acknowledgments

This research was funded by FCT (Fundação para a Ciência e Tecnologia), UIDB/04423/2020, UIDP/04423/2020, grant number 2021.06177.BD, through national funds.

References

1. Veiga-Matos, J. Remião, F. Morales, A. I. Pharmacokinetics and Toxicokinetics Roles of Membrane Transporters at Kidney Level. *J. Pharm. Pharm. Sci* **2020**, *23*, 333–356.
2. Silva, R. Palmeira, A. Carmo, H. Barbosa, D. J. Gameiro, M. Gomes, A. Paiva, A. M. Sousa, E. Pinto, M. Bastos, M. L. Remião, F. P-glycoprotein induction in Caco-2 cells by newly synthetized thioxanthones prevents paraquat cytotoxicity. *Arch. Toxicol* **2015**, *89*, 1783–1800.
3. Martins, E. Silva, V. Lemos, A. Palmeira, A. Puthongking, P. Sousa, E. Rocha-Pereira, C. Ghanem, C. I. Carmo, H. Remião, F. Silva, R. Newly synthesized oxygenated xanthones as potential P-glycoprotein activators: In vitro, ex vivo, and in silico studies, *Molecules* **2019**, *24, 0-27*.

Poster 55

Clinical and toxicological effects of GLP-1 agonists

M. J. Teixeira [1,2,*] and D. Dias da Silva [1,3,4]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences (IUCS), CESPU, 4585-116 Gandra, Portugal
[2] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[3] UCIBIO/REQUIMTE, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[4] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

* Correspondence: amariajsteixeira@gmail.com

https://doi.org/10.48797/sl.2023.97

Abstract

Background: Glucagon-like peptide-1 receptor (GLP-1) agonists have been investigated and applied for the treatment of type 2 diabetes mellitus (T2DM) and obesity due to their ability to increase glucose-dependent insulin secretion. However, due to their recent therapeutic use, less is known in what concerns the long-term toxicological effects of these medicines. **Objective:** Herein, we compiled the available information on the clinical and toxicological effects of GLP-1 agonists. **Methods:** A literature search was carried out in PubMed (U.S. National Library of Medicine) to find relevant articles dealing with the clinical and toxicology effects of GLP-1 agonists, without a limiting period and placing a special focus on clinical studies. **Results:** All GLP-1 agonists increase hyperglycaemia-induced insulin secretion, suppressing glucagon secretion in hyperglycaemia or euglycaemia, slowing down gastric empty, preventing large post-meal glycaemic increments, and reducing caloric intake and body weight [1]. In addition, GLP-1 agonists are claimed to have pleiotropic effects on the cardiovascular system, which might be of particular relevance for patients with T2DM and/or obese, as these individuals are at increased cardiovascular disease risk and display poorer recover from cardiovascular deleterious events, compared to controls [2]. GLP-1 agonists seem to have side effects on pancreas and thyroid, but current evidence does not show a cause-effect association between these drugs and the development of pancreatitis, pancreatic cancer, or thyroid cancer. The use of these drugs, mainly exenatide, has been associated with acute kidney injury, as well as local reactions in injection site [3]. **Conclusions:** GLP-1 agonists are a newly and widely recommended class of glucose-lowering agents with the ability to lower plasma glucose comparable to insulin regimens, but with a lower risk of hypoglycaemia and the added benefit of weight loss. More clinical trials and pharmacovigilance information are however needed to clarify the cardiovascular and overall safety profile of GLP-1 agonists.

Keywords: type-2 diabetes mellitus (T2DM); obesity; cardiovascular effects

Acknowledgments

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) in the scope of the projects UIDP/04378/2020 and UIDB/04378/2020 of the UCIBIO, and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB.

References

1. Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. Mol Metab. **2021**;46:101102.
2. Okerson T, Chilton RJ. The cardiovascular effects of GLP-1 receptor agonists. *Cardiovasc Ther.* **2012**;30(3):e146-55.
3. Filippatos TD, Panagiotopoulou TV, Elisaf MS. Adverse Effects of GLP-1 Receptor Agonists. *Rev Diabet Stud*. **2014**;11(3-4):202-30.

Poster 56

Does paraquat exposure affect peroxisomal enzyme activities in trout?

A. D. Resende [1,2,*], A. Lobo-da-Cunha [2,3] and E. Rocha [2,3]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[2] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), Terminal de Cruzeiros do Porto de Leixões, University of Porto, 4050-208 Matosinhos, Portugal
[3] ICBAS – School of Medicine and Biomedical Sciences, University of Porto, 4099-003, Porto, Portugal

* Correspondence: albina.resende@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.98

Abstract

Background: Environmental pollutants may exert toxicological effects in aquatic organisms, sometimes inducing the intracellular formation of reactive oxygen species with consequent cellular damage [1,2]. Paraquat is a potent herbicide that can be highly toxic for fish, causing morphological and biochemical alterations in several organs [3,4]. **Objective:** Since activity of peroxisomal enzymes is implicated in the reactive oxygen species and xenobiotic metabolisms [5], a subacute exposure of brown trout (*Salmo trutta* f. *fario*) to a waterborne relevant environmental concentration of paraquat was performed to verify if some peroxisomal enzymes, particularly from the liver and kidney, would be significantly altered. **Methods:** Immature (1-year-old) brown trouts were exposed to 0.3 mg/L of paraquat for 7 or 15 days; concentration was renewed every 2-3 days. Ten fish were collected on day 0 of the experience as control. At the end of the exposure period, ten animals from each group, paraquat and control, were collected. The catalase, D-amino acid oxidase, and urate oxidase enzyme activities were measured spectrophotometrically in liver and renal homogenates. Statistical analysis recurred to one-way ANOVA. **Results:** The liver's enzyme activities did not differ significantly between the control and paraquat groups after 7 and 15 days of exposure. In the kidney, urate oxidase was not detectable, and no statistical differences were found between the control and paraquat groups. During the experiment, however, both control and exposed groups showed increased catalase activity while decreasing D-amino acid oxidase activity. **Conclusions:** At the tested concentration, paraquat did not affect the studied peroxisomal enzymes in the liver and kidney. Notably, there were changes over time, warning that captivity or experimental stress influenced the enzyme activities. This aspect deserves further study. Moreover, research with other concentrations and targets should be done to refine the assessment of paraquat's toxicological potential for peroxisomes.

Keywords: herbicide; brown trout; enzyme activities; kidney peroxisomes; liver peroxisomes

Acknowledgments

This research was funded by Fundação para a Ciência e Tecnologia (FCT) for funds to CIIMAR (UIDB/04423/2020 and UIDP/04423/2020).

References

1. Badroo, I.A.; Nandurkar, H.P.; Khanday, A.H. Toxicological impacts of herbicide paraquat dichloride on histological profile (gills, liver, and kidney) of freshwater fish *Channa punctatus* (Bloch). *Environ Sci Pollut Res Int.* **2020**, *27*(31), 39054-39067.
2. Masters, C.; Crane, D. On the Role of the Peroxisome in Cell Differentiation and Carcinogenesis. *Molecular and Cellular Biochemistry* **1998**, *187*, 85-97.
3. Dinis-Oliveira, R.J.; Duarte, J.A.; Sánchez-Navarro, A.; Remião, F.; Bastos, M.L.; Carvalho, F. Paraquat Poisonings: Mechanisms of Lung Toxicity, Clinical Features, and Treatment. *Critical Reviews in Toxicology* **2008**, *38*:1, 13-71.
4. Sartori, F.; Vidrio, E. Environmental fate and ecotoxicology of paraquat: a California perspective. *Toxicological & Environmental Chemistry* **2018**, *100*:5-7, 479-517.
5. Farmen, E.; Olsvik, P.A.; Berntssen, M.H.; Hylland, K.; Tollefsen, K.E. Oxidative stress responses in rainbow trout (*Oncorhynchus mykiss*) hepatocytes exposed to pro-oxidants and a complex environmental sample. *Comp Biochem Physiol C Toxicol Pharmacol.* **2010**, *151*(4), 431-8.

Poster 57

Evaluation of the systemic oxidative stress status upon *in vivo* exposure to tramadol and tapentadol

C. Cardoso [1,*], R. J. Dinis-Oliveira [1,2,3,4], S. Leal [1,5], J. Barbosa [1,2,ǂ] and J. Faria [1,2,ǂ]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences–CESPU (IUCS-CESPU), 4585-116 Gandra, PRD, Portugal
[2] UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, 4099-002 Porto, Portugal
[4] MTG Research and Development Lab, 4200-604 Porto, Portugal
[5] CINTESIS@RISE, Department of Community Medicine, Information and Health Decision Sciences, Faculty of Medicine, University of Porto, 4099-002 Porto, Portugal
[ǂ] these authors contributed equally to this work

* Correspondence: cristianacardoso20@outlook.pt

https://doi.org/10.48797/sl.2023.99

Abstract

Background: Tramadol and tapentadol are synthetic centrally acting analgesic opioids, used in the treatment of moderate to severe pain [1]. Despite their optimized therapeutic and safety profiles, these compounds are associated with adverse effects, namely CNS and respiratory depression, abuse and dependence [2, 3]. Oxidative stress is one of the main toxicity mechanisms triggered by opioids [1, 4, 5]. **Objective:** The aim of this study was to evaluate putative systemic oxidative stress changes induced by a therapeutic dose of tramadol and tapentadol. **Methods:** Three groups of Wistar rats (9 animals each) were administered intraperitoneally with 50 mg/kg tramadol/tapentadol during 8 alternate days, while the control group was treated with saline solution [1]. Serum total antioxidant capacity and ROS/RNS levels were determined through spectrophotometry, whilst serum cysteine and homocysteine levels were quantified through ELISA, with commercial kits, according to the manufacturers' instructions. Statistical data analysis was performed through an Analysis of Variance (ANOVA), followed by Dunnett's multiple comparison's test. **Results:** An increase in ROS/RNS levels was observed in tramadol (*p<0.05) and tapentadol (***p<0.001) groups. However, regarding the antioxidant concentration, no significant differences were found. A statistically significant decrease in the concentration of cysteine was observed in the tramadol-administered group (*p<0.05). Furthermore, a statistically significant increase in the concentration of homocysteine was evident in the tapentadol-administered group (*p<0.05). **Conclusions:** The increase in ROS/RNS levels demonstrates that tramadol and tapentadol cause oxidative stress, with no changes in the total antioxidant capacity. However, as cysteine may have an antioxidant effect, the decrease in its serum levels may indicate that tramadol affects the levels of adjuvant antioxidants [6]. Since high levels of homocysteine causes oxidative stress, the increase in its serum concentration indicates that tapentadol induces oxidative stress [7]. In conclusion, tramadol and tapentadol must have a controlled prescription given their potential oxidative toxicity.

Keywords: tramadol; tapentadol; oxidative stress; cysteine; homocysteine

Acknowledgments

This research was funded by Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), project/grant CBToxAtOpi-GI2-CESPU-2022.

References

1. Barbosa, J., et al., Tramadol and Tapentadol Induce Conditioned Place Preference with a Differential Impact on Rewarding Memory and Incubation of Craving. Pharmaceuticals (Basel), **2023**. 16(1).
2. Beakley, B.D., A.M. Kaye, and A.D. Kaye, Tramadol, Pharmacology, Side Effects, and Serotonin Syndrome: A Review. Pain Physician, **2015**. 18(4): p. 395-400.
3. Polati, E., et al., Tapentadol: an overview of the safety profile. J Pain Res, **2019**. 12: p. 1569-1576.
4. Ali, H.A., et al., Neurotoxic, Hepatotoxic and Nephrotoxic Effects of Tramadol Administration in Rats. J Mol Neurosci, **2020**. 70(12): p. 1934-1942.
5. Faria, J., et al., Comparative study of the neurotoxicological effects of tramadol and tapentadol in SH-SY5Y cells. Toxicology, **2016**. 359-360: p. 1-10.
6. Chiang, F.F., et al., Cysteine Regulates Oxidative Stress and Glutathione-Related Antioxidative Capacity before and after Colorectal Tumor Resection. Int J Mol Sci, **2022**. 23(17).
7. Perna, A.F., D. Ingrosso, and N.G. De Santo, Homocysteine and oxidative stress. Amino Acids, **2003**. 25(3-4): p. 409-17.

Poster 58

Toxicity of amphetamine-type drugs in rat cardiomyocyte cells involves oxidative stress and the formation of acidic vesicular organelles

M. J. Valente [1], A. M. Araújo [2,*], F. Carvalho [3,4] and M. Carvalho [3,4,5,6]

[1] National Food Institute, Technical University of Denmark, 2800 Kongens Lyngby, Denmark
[2] LAQV, REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] UCIBIO/REQUIMTE, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4] Associate Laboratory i4HB, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[5] FP-I3ID, FP-BHS, University Fernando Pessoa, Porto, Portugal
[6] Faculty of Health Sciences, University Fernando Pessoa, Porto, Portugal

* Correspondence: amaraujo@ff.up.pt

https://doi.org/10.48797/sl.2023.100

Abstract

Background: Synthetic cathinones (SCs) are recreational psychoactive substances with pharmacological properties resembling those of classical amphetamines, such as 3,4-methylenedioxymethamphetamine (MDMA; *ecstasy*) [1]. Although the use of SCs has been linked to adverse health outcomes, including myocardial infarction and sudden cardiac deaths [2], the underlying cardiotoxic mechanisms are still unknown. **Objective:** This study evaluates the potential *in vitro* cardiotoxicity mechanisms of two commonly abused SCs, 3,4-methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxymethcathinone (methylone), and compares them with those obtained for MDMA. **Methods:** The H9c2 cell line was exposed for 24 hours to a wide range of concentrations (0.01-15 mM for MDPV; 0.01-20 mM for MDMA and methylone). The cytotoxic response was measured through the MTT assay and the role of oxidative stress was evaluated through the production of reactive oxygen and nitrogen species (ROS/RNS). The formation of acidic vesicular organelles (AVOs) was also evaluated by fluorescence microscopy in cells exposed to EC_{30} or EC_{60} of each drug. **Results:** All compounds decreased cell viability in a concentration-dependent manner. MDPV and MDMA were the most toxic drugs (EC_{50} 1.76, 1.86 mM, respectively), while methylone was the least cardiotoxic derivative (EC_{50} 3.30 mM; $p<0.0001$ *vs*. EC_{50} MDMA; $p<0.0001$ *vs*. overall fit MDMA). MDMA triggered ROS/RNS production only at 0.8 mM ($p<0.0001$ *vs*. control) and MDPV only at 1.6 and 3 mM ($p<0.01$ *vs*. control). In contrast, methylone demonstrated a significant increase for all concentrations between 0.05 mM ($p<0.0001$ *vs*. control) and 12 mM ($p<0.05$ *vs*. control). All drugs prompted the formation of AVOs in a concentration-dependent manner. **Conclusions:** Our findings are the first to show that SCs cause *in vitro* cardiotoxicity, and that oxidative stress and autophagy may play a role in these events. Further research is needed to explore the underlying molecular mechanisms.

Keywords: methylone; MDPV; MDMA; cardiotoxicity; oxidative stress

Acknowledgments

This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

References

1. Valente, M.J.; Guedes de Pinho, P. Khat and synthetic cathinones: a review. *Arch Toxicol* **2014**, 88, 15-45.
2. Radaelli, D.; Manfredi, A. Synthetic cannabinoids and cathinones cardiotoxicity: facts and perspectives. *Curr Neuropharmacol* **2021**, 19, 2038-2048.

Poster 59

Mechanisms behind the neurotoxicity of 2C-I and 25I-NBOMe drugs

E. Gil-Martins [1,2,*], F. Cagide [2], A. Borer [1], D. J. Barbosa [3,4], D. Martins [2], C. Fernandes [2], F. Remião [1], F. Borges [2] and R. Silva [1]

[1] UCIBIO – Applied Molecular Biosciences Unit, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[2] CIQUP-IMS, Research Center in Chemistry, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal
[3] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Avenida Central de Gandra 1317, 4585-116 Gandra, Portugal
[4] Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

* Correspondence: evagilmartins@gmail.com

https://doi.org/10.48797/sl.2023.101

Abstract

Background: New Psychoactive Substances (NPS) pose significant health and legal risks worldwide. At the end of 2021, the European Monitoring Centre for Drugs and Drug Addiction was monitoring 886 NPS, 106 of them phenethylamines [1]. Phenethylamine derivatives include 2,5-dimethoxyphenethylamine-based (2C) and *N*-benzylphenethylamine-based (NBOMe) drugs, widely known for their psychedelic effects. However, their toxicological profile remains poorly characterized [2,3]. **Objective:** To address this gap, 2C-I (2-(4-iodo-2,5-dimethoxyphenyl)ethanamine) and its corresponding NBOMe derivative (2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine) were synthesized and their neurotoxic profile evaluated, elucidating potential mechanistic pathways involved in drug-induced cytotoxicity. **Methods:** 2C-I and 25I-NBOMe were synthesized and structurally characterized by nuclear magnetic resonance and mass spectrometry techniques. Neuronal SH-SY5Y cells differentiated into a dopaminergic phenotype and primary rat cortical neurons, which were exposed to the drugs for 24 hours, were used for the *in vitro* experiments. Drugs' neurotoxicity and the impact of MAO-mediated inhibition on drug-induced cytotoxicity were evaluated using the neutral red uptake assay. The capacity of the drugs to generate free radicals was estimated using the DCFH-DA probe and their impact on the intracellular GSH and ATP levels were assessed using the DTNB-reductase-recycling and the ATP bioluminescence assays, respectively. Changes in the mitochondrial membrane potential were investigated using the JC-1 probe. The chromatographic hydrophobicity index (CHI) of the drugs was also evaluated by Fast-Gradient RP-HPLC. **Results:** Both drugs exhibited a concentration-dependent neurotoxic effect, with 25I-NBOMe being more cytotoxic than its counterpart, which supports the drugs' lipophilicity data. MAO inhibition had no significant impact on drug-induced cytotoxicity. No significant changes in ROS production were observed for both drugs, but a significant decrease in intracellular GSH and ATP levels, and significant mitochondrial membrane depolarization was detected. **Conclusions:** The introduction of a NBOMe substituent significantly increased all the evaluated neurotoxic effects, demonstrating the high potential of these drugs to induce severe adverse reactions.

Keywords: new psychoactive substances; neurotoxicity; 2C-I; 25I-NBOMe

Acknowledgments

This research was funded by the Foundation for Science and Technology (FCT, Portugal), in the scope of the projects UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO), LA/P/0140/2020 (i4HB), UIDB/00081/2020 (CIQUP), LA/P/0056/2020 (IMS) and by FEDER/COMPETE POCI-01-0145-FEDER-02839. Eva Gil-Martins (SFRH/BD/146527/2019), Daniel Martins (PD/BD/135122/2017), Carlos Fernandes (2021.04016.CEECIND) and Fernando Cagide (SFRH/BDP/72923/2010) were supported by FCT fellowships from FCT.

References

1. European Monitoring Centre for Drugs and Drug Addiction, European Drug Report **2022**: Trends and Developments, Publications Office of the European Union, Luxembourg, **2022**; pp. 40.
2. Shulgin, A.; A. Shulgin. PIHKAL: A Chemical Love Story; Transform press: Berkeley, California, United States of America, **1991**.
3. Nichols, D. E. Psychedelics. Pharmacol Rev **2016**, 68.2, 264-355.

Poster 60

The synthetic cannabinoids ADB-FUBINACA and AMB-FUBINACA accelerate SH-SY5Y proliferation via stimulation of CB1 and CB2 cannabinoid receptors

D. Dias-da-Silva [1,2,3,*], R. Roque-Bravo [1,2], J. P. Silva [1,2], H. Carmo [1,2] and F. Carvalho [1,2]

[1] UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[2] Associated Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, Advanced Polytechnic and University Cooperative (CESPU), CRL, 4585-116 Gandra, Portugal

* Correspondence: diana.dias@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.102

Abstract

Background: Synthetic cannabinoids (SC), one of the most popular groups of new psychoactive substances, display a broad pharmacological action close to that of Δ^9-tetrahydrocannabinol (THC). However, while THC is a partial agonist of the cannabinoid receptors type 1 and 2 (CB1 and CB2, respectively), SC present a full and more potent agonistic activity on these receptors [1]. Since new evidence demonstrates that cannabis can accelerate ageing/cell senescence [2,3], we therefore hypothesized that SC might also display this ability. **Objective:** To measure putative SC-induced acceleration of neuronal senescence and, in case of positive effects, to ascertain the extent to which SC-mediated proliferation depends on the effects of the CB1 and CB2. **Methods:** This work began by evaluating neuronal proliferation of SH-SY5Y human neuroblastoma cells after exposure to two trendy SC, ADB-FUBINACA and AMB-FUBINACA. Proliferation was evaluated using the sulforhodamine B (SRB) assay, following exposure to drugs at the biologically-relevant concentrations of 1µM, 1nM and 1pM, for 24h, 48h, 72h, and 96h. Then cells were incubated with 0.5 µM SR141716A and SR144528 (selective inverse agonists for CB1 and CB2, respectively), for 20 min prior to exposure to the SC. **Results:** At 96h, both ADB-FUBINACA and AMB-FUBINACA significantly increased SH-SY5Y proliferation ($p<0.05$) at all tested concentrations (1µM, 1nM and 1pM, respectively: 119%, 125%, and 129% for ADB-FUBINACA; 129%, 140%, and 140% for AMB-FUBINACA), compared to the control. Co-exposure of these SC and the receptor inverse agonists reverted the proliferation increase to values not significantly different from that of the antagonist controls, indicating that CB1 and CB2 are likely involved in this SC-mediated proliferative effect. **Conclusions:** SH-SY5Y proliferation was accelerated after SC exposure. As this increased proliferation results in increased cell divisions, our data suggest that the SC tested may accelerate senescence-related processes. As such, assessing key senescence markers will ensue.

Keywords: cell senescence; ADB-FUBINACA; AMB-FUBINACA

Acknowledgments

This work was supported by FCT — Fundação para a Ciência e a Tecnologia (projects UIDP/04378/2020 and UIDB/04378/2020 of the Applied Molecular Biosciences—UCIBIO), and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB. RRB and JPS are supported by the PhD grant 2020.04493.BD and research contract (under Scientific Employment Stimulus) 2021.01789.CEECIND, respectively.

References

1. Roque Bravo, R.; Silva, R. S. Synthetic Cannabinoids: A Pharmacological and Toxicological Overview. *Annu Rev Pharmacol Toxicol.* **2023**; 63(1).
2. Burggren, A.C.; Siddarth, P. Subregional Hippocampal Thickness Abnormalities in Older Adults with a History of Heavy Cannabis Use. *Cannabis Cannabinoid Res*. **2018***; 3*(1): 242-51
3. Allen, J.P.; Danoff, J.S. Lifetime marijuana use and epigenetic age acceleration: A 17-year prospective examination. *Drug Alcohol Depend*. **2022***; 233*, 109363.

Poster 61

Assessment of the CYP450 inhibitory potential of LSD, 5-MeO-DMT and mescaline: an *in vitro* study

M. Silva-Carvalho [1,*], A. M. Brito-da-Costa [1,2,3], R. J. Dinis-Oliveira [1,2,4], Á. Madureira-Carvalho [1,5] and D. Dias da Silva [1,2,3]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences (IUCS) – CESPU, Gandra, Portugal
[2] UCIBIO/REQUIMTE, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] Associate Laboratory i4HB – Institute of Health and Bioeconomy, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[5] LAQV/REQUIMTE, Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Porto, Porto, Portugal

* Correspondence: a29632@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.103

Abstract

Background: LSD, 5-MeO-DMT and mescaline are classic hallucinogens known for their recreational use, whose consumption increased in the last decades. Despite some available data on the toxicokinetics of these drugs, little is known about their CYP450 metabolism [1,2,3]. Nevertheless, this information is of crucial relevance to predict drug-drug interactions and understand toxicological phenomena, in particular interindividual variability. **Objective:** This study evaluated the potential inhibition of LSD, 5-MeO-DMT and mescaline over CYP450 isoenzymes (CYP3A4, CYP2D6, CYP2B6 and CYP2A6). **Methods:** The Vivid® CYP450 screening kits were used following the manufacturer's instructions. Concentration ranges tested for each drug were $6.1x10^{-5}$–1.0 mM, $1.95x10^{-4}$–4.0 mM and $6.1x10^{-5}$–1.0 mM for CYP3A4; $9.54x10^{-8}$–1.0 mM, $9.54x10^{-7}$–4.0 mM and $6.1x10^{-5}$–4.0 m for CYP2D6; $2.56x10^{-5}$–2.0 mM, $2.44x10^{-4}$–6.0 mM and $6.1x10^{-5}$–4.0 mM for CYP2B6; and $1.91x10^{-6}$–1.0 mM, $2.86x10^{-6}$–4.0 mM and $2.29x10^{-5}$–1.0 mM for CYP2A6, for LSD, 5-MeO-DMT and mescaline, respectively. Solvent and positive controls of inhibition, i.e., ketononazole (CYP3A4), quinidine (CYP2D6), miconazole (CYP2B6) and tranylcypromine (CYP2A6) were used. Fluorescence was measured for 60 minutes at excitation and emission wavelengths of 415/20 and 460/20 nm, respectively. The half-maximal inhibitory concentration (IC_{50}) was calculated using GraphPad Prism 9.3.0. Five independent experiments were performed for CYP3A4, four for CYP2D6 and two for CYP2B6 and 2A6. **Results:** IC_{50} values of 80.92 µM, 203.27 µM, 97.59 µM for CYP3A4; 0.61 µM, 3.47 µM, 558.53 µM for CYP2D6; 604.68 µM, 653.55 µM, 323.98 µM for CYP2B6; and 54.44 µM, 124.82 µM, 96.35 µM for CYP2A6, were obtained for LSD, 5-MeO-DMT and mescaline, respectively. **Conclusions:** LSD and 5-MeO-DMT have a strong potential to inhibit CYP2D6, which is highly polymorphic and therefore implicated in great toxicological interindividual variability. CYP3A4 which is involved in the metabolism of many drugs and food is also greatly inhibited by LSD and mescaline.

Keywords: hallucinogens; toxicokinetics; psychoactive substances

Acknowledgments

This research was funded by i) FCT — Fundação para a Ciência e a Tecnologia in the scope of the PhD grant 2021.04999.BD, the projects UIDP/04378/2020 and UIDB/04378/2020 of the UCIBIO, and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB; ii) IINFACTS-CESPU in the scope of the project PsiloPharma_PI2RL_IINFACTS_2021; and iii) EUROX Pharma.

References

1. Dinis-Oliveira RJ, Pereira CL and Dias da Silva D. Pharmacokinetic and Pharmacodynamic Aspects of Peyote and Mescaline: Clinical and Forensic Repercussions. Curr Mol Pharmacol 12:184-194, 2019
2. Holze F, Liechti ME, Hutten N, Mason NL, Dolder PC, Theunissen EL, Duthaler U, Feilding A, Ramaekers JG and Kuypers KPC. Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide Microdoses in Healthy Participants. Clin Pharmacol Ther 109:658-666, 2021
3. Jiang XL, Shen HW, Mager DE and Yu AM. Pharmacokinetic interactions between monoamine oxidase A inhibitor harmaline and 5-methoxy-N,N-dimethyltryptamine, and the impact of CYP2D6 status. Drug Metab Dispos 41:975-986, 2013

Poster 62

Sex estimation using canines' maximum length and width: a preliminary study in orthopantomograms

C. Sousa-Matos [1,*] and I. M. Caldas [1,2,3]

[1] TOXRUN - Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[2] Faculty of Dental Medicine, University of Porto, Rua Dr. Manuel Pereira da Silva, 4200-393 Porto, Portugal
[3] Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

* Correspondence: a28113@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.104

Abstract

Background: Sex estimation is fundamental in the forensic context, as it reduces the number of possibilities identity to a half [1]. In this field, several dental methods can be used, as teeth are very resistant structures and can provide valuable data regarding this estimation [2]. Canines have been described as the most dimorphic teeth in human dentition [3,4]. **Objective:** To aid in the forensic sex estimation process by adding new data considering canines' measurements performed in radiographs. **Methods:** Image J was used to measure canines' maximum length and width in 30 orthopantograms (opts), in pixels, from the clinical services of the Faculty of Dental Medicine, University of Porto. To minimize distortion, the ratio between length and width was analyzed. SPSS version 28.0 was used for data analysis. Categorical variables were described using frequencies and percentages, whereas continuous variables were described using maximal and minimal values, the mean and standard deviation. Mean values of the different ratios were compared, by sex, using the independent samples t-test. Statistical significance was set at 5%. **Results:** Most opts belonged to male subjects (n=17, 56.7%). Males displayed superior ratio mean values in all canines, which ranged from 3.59 to 3.62, whereas, in females, values ranged between 3.38 and 3.52. Regardless of the canine (upper, lower, right or left) no differences were found in the mean ratio between sexes (p>0,05). **Conclusions:** Although male displayed bigger mean ratio in all canines, these differences do not seem to be enough for sex estimation. It is possible that different results may be achieved if the sample is increased.

Keywords: forensics; biological profile; teeth; odontometrics

Acknowledgments

This research received no external funding.

References

1. Jayakrishnan, J.M.; Reddy, J.; Vinod Kumar, R.B.. Role of forensic odontology and anthropology in the identification of human remains. *J Oral Maxillofac Pathol* **2021**, 25,: 543-547.
2. Prabhu, S.; Acharya, A.B.. Odontometric sex assessment in Indians. *Forensic Sci Int* **2009,**192: 129, e121-5.
3. Aggarwal, B.; Gorea, R.K.; Gorea, A.. Comparative analysis of clinical and experimental methods for determination of sexual dimorphism of mandibular canines. *J Forensic Leg Med* **2016**, 44, 20-23.
4. Schwartz, G.T.; Dean, M.C. Sexual dimorphism in modern human permanent teeth. *Am J Phys Anthropol* **2005**,128, 312-317.

Poster 63

Graphical abstracts in Forensic Sciences – science communication and literacy

C. Florek [1], R. J. Dinis-Oliveira [2,3,4] and J. Soares Carrola [5,6,*]

[1] Department of Vernacular Letters (DLV), Federal University of Santa Maria, Rio Grande do Sul, Brazil
[2] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences (IUCS) – CESPU, Gandra, Portugal
[3] UCIBIO/REQUIMTE, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[5] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[6] Inov4Agro, Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, Vila Real, Portugal

* Correspondence: joao@utad.pt

https://doi.org/10.48797/sl.2023.105

Abstract

Background: Scientific digital communication presents a diversity of add-on summaries to verbal abstracts [1]. Among these native HTML and PDF publications, Graphical Abstracts (GAs) are possibly the oldest [2] and are prevalent in chemistry, medicine, and biology [3]. Even so, some biomedical sub-areas seem to resist to the use of GAs, including the Forensic area. **Objective:** Review the frequency of GAs in scientific forensics journals and alert for the importance of at least optional GA presentation. **Methods:** A search of scientific literature was done using *Pubmed* and *ScienceDirect* looking for: "graphical abstract", "forensic" and "forensic science", and analyzing major journals assessing the prevalence of GA: mandatory; optional; specific image; the first image of the paper; only online or in the pdf. **Results:** According to the h5-index, eight publishers are responsible for publishing the 20 most important Forensic Sciences journals. GAs are optional in 30% (n=6) and mandatory in 5% (n=1), some use the first image as GA. In Elsevier, Oxford Academy and MDPI journals, GAs are mandatory or optional. Guidelines for GA elaboration are usually brief, and Elsevier presents more detailed guidelines. Bentham Science asks for animated video abstract (as a complement to GA), the most complex format to be produced. **Conclusions:** The reduced adherence of important publishers to GAs may be related to I) idiosyncrasies from forensic areas which can make GAs unattractive; II) resistance to digital genres [4,5]; III) being seen by others as means of trivializing and simplifying technical knowledge [6,7]. Furthermore, for some researchers, GA construction is time-consuming and a waste of time. We can deduce also that a lack of literacy is a possible factor. It is essential to focus on critical digital literacy strategies to prepare students and researchers to produce high-quality multimodal genres with diverse digital tools without losing scientific quality.

Keywords: forensic sciences; graphical abstract; critical digital literacy; science communication

Acknowledgments

This work was supported by Portuguese National Funds by Foundation for Science and Technology (FCT), UIDB/04033/2020 (CITAB/Inov4Agro).

References

1. Katsampoxaki-Hodgetts, K. The emergence of a new inclusive meta-scientific genre: 'the Bigger Picture' Journal of *English for Academic Purposes* **2022**, *57*.
2. Pérez-Llantada, C. The article of the future: strategies for genre stability and change. *English for Specific Purposes* **2013**, *32*, 221-235.
3. Hendges, G. R.; Florek, C. S. The graphical abstract as a new genre in the promotion of science. In *Science communication on the internet: old genres meet new genres*. Luzón, M., Pérez-LLantada, C., Eds. Johns Benjamins: Amsterdam, Philadelphia, **2019**. pp. 59-79.
4. Luzón, M. J. Connecting genres and languages in online scholarly communication: An analysis of research group blogs. *Written Communication*, **2017,** *34.*
5. Luzón, M. J. Features of online ELF in research group blogs written by multilingual Scholars. *Discourse, Context & Media* **2018**, 24, pp. 24-32.
6. Rowley-Jolivet, E.; Carter-Thomas, S. Scholarly soundbites Audio Visual innovations in digital science and their implications for genre evolution. In *Science communication on the Internet: Old genres meet new genres*. Luzón, C. Pérez-Llantada, Eds. John Benjamins: Amsterdam, The Netherlands, pp. 81-106.
7. Sancho Guinda, C. Scientific stylisation or the 'democracy dilemma' of graphical abstracts. *Publications*, **2022**, 10.

Poster 64

Gold nanoparticles for forensic application

A. C. Almeida Cardoso da Costa [1,*], A. Mateus [1] and T. M. Fernandes Patrício [1]

[1] CDRSP – Center for Rapid and Sustainable Product Development, Rua de Portugal, Marinha Grande, Portugal

* Correspondence: ana.catarina.alcc@gmail.com

https://doi.org/10.48797/sl.2023.106

Abstract

Background: Nanotechnology has grown exponentially over the years, considering the versatility of applications [1]. Metallic nanoparticles present various advantages, highlighting their versatility, low toxicity, high biocompatibility, biodegradability, stability, and low production cost [2–4]. Metallic nanoparticles, such as gold nanoparticles (NPAu), can be used for incorporation in systems due to the colorimetric properties that allow identification through color change [1]. There are methodologies for detecting latent fingerprints under development, using NPAu for forensic application [2–4]. On the other hand, NPAu are used for illicit drug testing methods [2–4]. One of the barriers to the application of metallic nanoparticles is their synthesis through manual methodologies, making this process time-consuming and complex. The growing interest in nanoparticles and biomaterials opens the opportunity to design and develop novel and optimized prototype for biomaterials and nanoparticles synthesis. **Objective:** Demonstrate the potential of applying a versatile prototype for the synthesis of nanoparticles and biomaterials for biomedical and forensic applications. **Methods:** Herein, a versatile prototype for the synthesis of biomaterials and nanoparticles, namely NPAu is presented [5]. The main aim is to synthesize gold nanoparticles under controlled conditions, as temperature, pH, conductivity, among others. The as-produced nanoparticles will be used to identify diverse diseases, applied to biomedical or forensic applications. **Results:** A versatile prototype for biomaterials synthesis is presented. Gold nanoparticles will be synthesized, and chemical, physical, thermal properties will be evaluated. A proper biological condition will be addressed to characterize the gold nanoparticles. NPAu will be tested for the detection of traces, drugs or diseases, to be for example applied to several forensic areas. **Conclusions:** Development of the BioRobotBeads system demonstrates an emergent evolution of nanotechnology, especially for biomedical and forensic applications.

Keywords: prototype; nanoparticles; forensic applications

Acknowledgments

This work was financially supported by the Fundação para a Ciência e a Tecnologia FCT/MCTES (PIDDAC) through the following Projects: UIDB/04044/2020; UIDP/04044/2020; Associate Laboratory ARISE LA/P/0112/2020; PAMI - ROTEIRO/0328/2013 (Nº 022158); PTCentroDiH (03/C16-i03/2022-768) (POCI-01-0247-FEDER-047081).

References

1. Mohamed, A.A. Gold is going forensic. *Gold Bull* **2011**. 44, 71–77.
2. Klębowski, B.; Depciuch, J.; Parlińska-Wojtan, M.; Baran, J. Applications of noble metal-based nanoparticles in medicine. *Int J Mol Sci* **2018**, 19, 4031.
3. Salata, O. Applications of nanoparticles in biology and medicine. *J Nanobiotechnology* **2004**, 2, 3.
4. Alaribe, F.N.; Manoto, S.L.; Motaung, S.C.K.M. Scaffolds from biomaterials: advantages and limitations in bone and tissue engineering. *Biologia (Bratisl)* **2016**, 71, 353-366.
5. Comune, M.; Rai, A.; Palma, P.; TondaTuro, C.; Ferreira, L. Antimicrobial and pro-angiogenic properties of soluble and nanoparticle-immobilized LL37. *Biomater Sci* **2021**, 9, 8153-8159.

Poster 65

Variation of the physical-chemical parameters of diverse water bodies for study of diatom distribution and composition for forensic investigations

M. Portugal [1], C. Couto [1,2,*], A. Madureira-Carvalho [1,3] and C. Ribeiro [1]

[1] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[2] LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] LAQV/REQUIMTE, Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Porto, Portugal

* Correspondence: cristina.couto@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.107

Abstract

Background: Diatoms are microalgae of fundamental ecological role in aquatic ecosystems; however, these microorganisms have been shown to provide valuable information for forensic investigations. Due to the characteristic diatom distribution in a water body, the presence of diatoms in objects may allow to correlate to a specific aquatic system and/or a suspect. Additionally, in cases of suspicious of death by drowning, the presence of diatoms in some organs and/or bones may give important information to support confirmation [1-3]. The occurrence of diatoms, concerning diversity and frequency in a water body, depends on temporal and physical-chemical water parameters [3,4]. **Objective:** The aim of this study was to determine the seasonal and spatial variation of the physical-chemical parameters of different water for further correlation with the presence and geo-temporal variation of diatom composition. **Methods:** Six sampling points were selected in different regions of the Porto District: two wells (Póvoa de Varzim and Paredes); two on the Asprela streams; two in the natural reserve area of the Ave River. Samples were collected seasonally (Summer, Autumn and Winter) and temperature, conductivity, turbidity, pH, dissolved oxygen, nitrate, nitrite and phosphate were measured. Water samples aliquots were separated for further analysis for diatom composition. **Results:** The results showed a seasonal and spatial variation of physical-chemical water parameters. High levels of turbidity were found in autumn, in all water bodies and the sampling point located in natural reserve showed the highest value (589.6 NTU), and the highest content of nutrient which may affect diatom composition. Preliminary studies showed a low occurrence of diatom in well water samples. **Conclusions:** These results suggest that, for these water bodies, the presence of suspended diatom may be low which may difficult the use of these organisms for forensic investigation. Nevertheless, further experiments are ongoing to correlate physical-chemical parameters with composition of other water bodies.

Keywords: diatoms; physical-chemical parameters; well; river; streams

Acknowledgments

This research received no external funding.

References

1. Smol, J. P.; Stoermer, E. F. *The diatoms applications for the environmental and earth sciences*. 2nd ed.; Cambridge University Press: Cambridge; New York; Melbourne; Madrid; Cape Town; Singepore; São Paulo; Dehli; Dubai; Tokyo; Mexico City, 2015.
2. Lunetta, P.; Miettinen, A.; Spilling, K.; Sajantila, A. False-positive diatom test: A real challenge? A post-mortem study using standardized protocols. *Legal Medicine* **2013**, 15, 229-234.
3. Seckbach, J.; Gordon, R. *Diatoms: fundamentals and applications*. Publisher: Wiley; Salem, Massachusetts, 2019.
4. Luís, A. T.; Durães, N.; de Almeida, S. F. P.; da Silva, E. F. Integrating geochemical (surface waters, stream sediments) and biological (diatoms) approaches to assess AMD environmental impact in a pyritic mining area: Aljustrel (Alentejo, Portugal). *J Environ Sci* **2016**, 42, 215-226.

Poster 66

Sexual harassment and burnout in health care context

J. Figueira [1,2,*] **and M. J. Alves** [1,2]

[1] Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), CRL, 4585-116, Gandra, Portugal
[2] TOXRUN - Unidade de Investigação em Toxicologia, Instituto Universitário de Ciências da Saúde (IUCS), CESPU), CRL, 4585-116, Gandra, Portugal

* Correspondence: a32989@alunos.cespu.pt

https://doi.org/10.48797/sl.2023.108

Abstract

Background: Burnout is a major problem among physicians [1]. Women physicians experience higher rates of both burnout and sexual harassment than their male counterparts. Sexual harassment may contribute to burnout. Some studies have shown a correlation between sexual harassment at workplace and burnout in women physicians [1]. **Objective:** The purpose of this study is to identify the relationship between sexual harassment and burnout in female physicians in a health care context. It is hypothesized that sexual harassment would be associated with female physician burnout. **Methods:** Apply a cross-sectional self-report study to assess burnout and sexual harassment among medical professionals [2]. Burnout will be assessed by the Maslach Burnout Inventory (MBI) for Portuguese samples. Sexual harassment will be assessed with an adaptation of the Sexual Experiences Survey – Victimization Form [3]. **Results:** It is expected that respondents report some kind of sexual harassment experiences perpetrated by their work colleagues and eventually those experiences would be significantly correlated with burnout scores on female physicians [1,4]. **Conclusions:** This study expects to find that reports of burnout and sexual harassment perpetrated by work colleagues are significantly correlated. The results may also imply that there are significant rates of sexual harassment in medical settings.

Keywords: sexual harassment; burnout; health care; physicians

Acknowledgments

This research received no external funding.

References

1. Mathews, E.; Hammarlund, R.; Kullar, R.; Mulligan, L.; Le, T.; Lauve, S.; Nzodom, C.; Crapanzano, K. Sexual harassment in the house of medicine and correlations to burnout: a cross-sectional survey. *Ochsner J* **2019**, *19*, 329-339.
2. Fonte, C. Adaptação e validação para português do questionário de Copenhagen Burnout Inventory (CBI). [Dissertação de Mestrado, Faculdade de Economia da Universidade de Coimbra]. **2011**.
3. Freitas, S. Vitimização sexual: estudo exploratório acerca da satisfação sexual e satisfação com a vida em estudantes universitárias. [Dissertação de Mestrado, Faculdade de Psicologia e de Ciências da Educação da Universidade do Porto]. **2020**.
4. Hu, Y.; Ellis, R.; Hewit, D.; Yang, A.; Cheung, E.; Moskowitz, J.; Potts, J.; Buyske, J.; Hoyt, D.; Nasca, T.; Bilimoria, K. Discrimination, abuse, harassment, and burnout in surgical residency training. *N Engl J Med* **2019**, 381, 1741-1752.

Poster 67

Sodium nitrite poisoning: an emerging trend in Forensic Toxicology

B. Senra [1,*]

[1] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

* Correspondence: up201906416@up.pt

https://doi.org/10.48797/sl.2023.109

Abstract

Background: Sodium nitrite ($NaNO_2$) is an odorless, white crystalline powder soluble in water, and like common salt in appearance and taste. It can be toxic for humans and can cause methemoglobinemia [1]. Its mechanism of toxicity mainly consists in the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) of one of the four heme structures in hemoglobin [2]. This is a growing topic due to the consecutive increase in the number of reported intoxication cases in recent years, mainly of suicide attempts by ingesting this powder. **Objective:** This study aims to summarize and characterize intentional and accidental sodium nitrite intake cases in what concerns to age, gender, and outcome (in particular mortality). **Methods:** A literature search was carried out on January 3, 2023, on PubMed. Only articles published in the last 5 years from the date of the search were selected. After excluding duplicate, off-topic, or no-access articles, 23 articles were selected, including 8 case reports, 8 case series and 6 review articles. **Results:** Of the 34 victims reported in the articles studied, 21 were male and 13 are female. The age range of the victims was from 16 to 70 years. 29 cases had an intentional character, while only 5 were caused by food poisoning. The amount of $NaNO_2$ ingested was from 0.75 to 113 g. **Conclusions:** A patient presenting with cyanosis and unresponsive without respiratory disease should raise suspicions of sodium nitrite poisoning. There was a higher mortality rate for older victims, so age should be a conditioning factor for the victim survival/death [1]. In 48.3% of the cases, $NaNO_2$ was obtained from the internet and online suicide forums. Thus, there should be limitation of this information on the internet and more control on $NaNO_2$ sales. These measures are being implemented in some countries, such as Canada [3].

Keywords: sodium nitrite; suicide attempts; methemoglobinemia; food poisoning

Acknowledgments

This research received no external funding.

References

1. Cvetković, D.; Živković, V.; Lukić, V.; Nikolić, S. Sodium nitrite food poisoning in one family. *Forensic Sci Med Pathol* **2019**, 15(1), 102-105.
2. Bugelli, V.; Tarozzi, I.; Manetti, A.C.; Stefanelli, F.; Di Paolo, M.; Chericoni, S. Four cases of sodium nitrite suicidal ingestion: A new trend and a relevant Forensic Pathology and Toxicology challenge. *Leg Med (Tokyo)* **2022**, 59, 102146.
3. Saleh, D.; Lucyk, S.; McGillis, E. Methemoglobinemia caused by sodium nitrite overdose. *Cmaj* **2022**, 194(30), E1066-e1067.

Poster 68

Shedding light on Portugal's first major forensic case: elemental analysis of biological samples 132 years later

Â. Silva-Bessa [1,2,3], R. Azevedo [4], A. Almeida [4] and R. J. Dinis-Oliveira [2,3,5,6,*]

[1] University of Coimbra, Centre for Functional Ecology, Laboratory of Forensic Anthropology, Department of Life Sciences, Coimbra, Portugal
[2] UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
[4] LAQV-REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[5] Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal
[6] MTG Research and Development Lab, Porto, Portugal

* Correspondence: ricardo.dinis@iucs.cespu.pt

https://doi.org/10.48797/sl.2023.110

Abstract

Background: In the late 19th century, Vicente Urbino de Freitas was accused of the death of his nephew, Mário Guilherme Augusto de Sampaio, and his brother-in-law, José António de Sampaio Junior [1-3]. Despite the controversy at the time surrounding the testimonies, autopsy reports and toxicological analyses carried out, Urbino de Freitas was found guilty. After an extensive research and consolidation of all historical records, Sampaio Junior's body was found buried at the Cemetery of Agramonte, Porto, in 2020. Permission for a new autopsy was granted, and biological samples were collected for further analysis. **Objective:** The main objective of this study was to perform an elemental analysis of the deceased's biological samples aiming to find additional relevant information regarding the possible cause-of-death, 132 years later. **Methods:** A total of thirteen freeze-dried soft and hard tissues (0.4-0.5 g) were mineralized using a microwave-assisted acid digestion procedure. The concentration of twenty-seven trace elements was determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using a Thermo Fisher Scientific iCAP™Q instrument (Waltham, MA, USA). Data analysis was performed using the R programming language. Here we present and discuss the results for Li, Fe, Co, Ni, Cu, Zn, Hg, and Pb. **Results:** Overall, the concentration of elements differed considerably according to the analysed specimen. Fe and Pb concentrations varied from 0.20 to 1.02 mg/g (mean=0.50; SD=0.29) and from 0.63 to 52.6 mg/g (mean=8.7; SD=14.3), respectively. **Conclusions:** This study provides important information on the levels of various metals in the cadaveric remains of a famous and old forensic case. However, this work has some limitations associated with the degradation of the biological materials and their possible postmortem contamination, mainly due to prolonged exposure to the coffin. Further analyses are needed and will be conducted on teeth due to their lower susceptibility to postmortem exchanges with the surrounding environment.

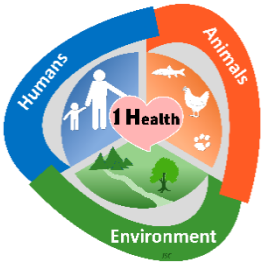
Keywords: Flores Street crime; José António de Sampaio Junior; exhumation; autopsy; trace elements

Acknowledgments

The first author was financed by FCT, grant number SFRH/BD/143242/2019.

References

1. Dinis-Oliveira, R.J. Portugal's first major forensic case and the genesis of forensic toxicology: 10 years of research to reconstruct the event. *Forensic Sci. Res.* **2019**, 4(1), 69-81.
2. Dinis-Oliveira, R.J. Analysis of the testimonial evidence of Portugal's first major forensic case: part II. *Forensic Sci. Res.* **2020**, 5(4), 266-285.
3. Dinis-Oliveira, R.J. Analysis of the autopsy, toxicological, and psychiatric reports of Portugal's first major forensic case: part III. *Forensic Sci. Res.* **2021**, 6(3), 250-272.



II TOXRUN INTERNATIONAL CONGRESS 2023

NO BOUNDARIES FOR TOXICOLOGY:
ONE HEALTH, ONE SOCIETY, ONE PLANET

27-28 APRIL, 2023 | PORTO, PORTUGAL

SPONSORS

SPONSORS

Thank you to all our Sponsors and Partners for their support and for contributing to the success of the II TOXRUN International Congress.

ORGANIZED BY

TOXRUN TOXICOLOGY RESEARCH UNIT

CESPU INSTITUTO UNIVERSITÁRIO DE CIÊNCIAS DA SAÚDE

IN PARTNERSHIP WITH

ORDEM DOS FARMACÊUTICOS 1835

avextra

Albert. Labs

EnantioTox

PTDC/CTA-AMB/6686/2020 FCT

SL Scientific Letters

SUPPORTED BY

CESPU COOPERATIVA DE ENSINO SUPERIOR POLITÉCNICO E UNIVERSITÁRIO

CESPU FORMAÇÃO

GI2 GABINETE PARA A INVESTIGAÇÃO & A INOVAÇÃO

ThermoFisher SCIENTIFIC

alfagene

Palex Constant Improvement LUSOPALEX

1692 TAYLOR FLADGATE TAYLOR'S

Fama LOW COST RNAVT Nº 3831

Numatic

III TOXRUN INTERNATIONAL CONGRESS 2024

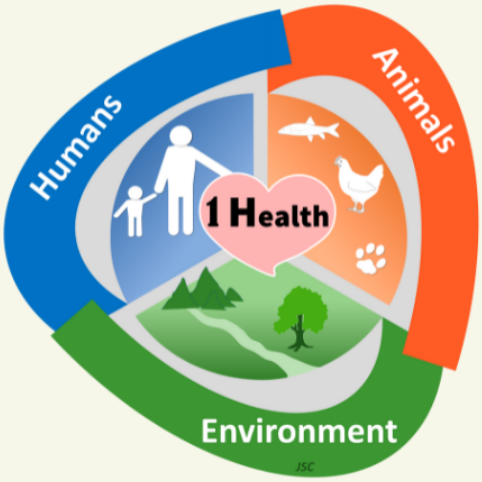
NO BOUNDARIES FOR TOXICOLOGY:
ONE HEALTH, ONE SOCIETY, ONE PLANET
THE BIG CHALLENGES OF THE 21ST CENTURY

2ND – 3RD OF MAY'24
PORTO / PORTUGAL

TOXRUN
TOXICOLOGY RESEARCH UNIT

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE

CESPU



II TOXRUN INTERNATIONAL CONGRESS 2023
No Boundaries for Toxicology: One Health, One Society, One Planet
27-28 April, 2023 | Porto, Portugal

BOOK OF ABSTRACTS

IUCS-CESPU Publishing

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE

Rua Central de Gandra, 1317, 4585-116, Gandra PRD, Portugal
Phone: +351 – 224157186