Scientific Letters

Poster Communication 74

Intracellular pH profile of platelets as a potential biomarker of postmortem interval: preliminary results

Paulino D Ndunduma[1,2], Carolina Cordeiro[1,2], Susana Coimbra[2,3,4], M Begoña Criado[1,2], M Céu Monteiro[1,2]

[1] UCIBIO – Applied Molecular Biosciences Unit, Toxicologic Pathology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal
[2] Associate Laboratory i4HB – Institute for Health and Bioeconomy, University Institute of Health Sciences – CESPU, 4585-116 Gandra, Portugal
[3] UCIBIO i4HB, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[4] UCIBIO, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), Avenida Central de Gandra 1317, 4585-116 Gandra, Portugal

* Correspondence: a40311@alunos.cespu.pt

Abstract

Background: One of the major challenges in forensic science is the precise determination of the postmortem interval (PMI). Several methods have been proposed to estimate PMI; however, many of them present significant limitations and large margins of error. Consequently, the development of more accurate and reliable approaches remains a major challenge in forensic science [1]. In this context, blood biomarkers have attracted increasing attention as potential tools for improving PMI estimation [2]. As platelets respond rapidly to changes in the microenvironment, leading to the production of metabolites that can modify their intracellular pH (pHi), postmortem assessment of the pHi profile of platelets promises to be a useful tool for PMI determination. **Objective**: To validate an experimental flow cytometry assay for platelet pHi determination. **Methods**: The BCECF-AM (2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester) fluorescent probe was used [3]. This probe emits green or yellow fluorescence depending on the pH value. The test was performed on whole blood (anticoagulant: sodium citrate) diluted in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer. Ammonium chloride (10 mM) and sodium propionate (100 mM) were used as controls for intracellular acidification and alkalinization. **Results**: The concentration of the fluorescent probe was optimized, obtaining values between 2mM and 4mM. Subsequently, kinetic assays were performed to evaluate the effect of pHi modifying agents on blood platelets. The assays showed an increase in probe fluorescence with ammonium chloride and a decrease with sodium propionate. **Conclusions**: Flow cytometry revealed to be a suitable methodology for analyzing platelet pHi. Indeed, the experimental conditions tested made it possible to detect differences in the pHi of blood platelets induced by acidifying and alkalizing agents. These preliminary findings provide a solid foundation for further research; *in vitro* and *vivo* studies, assessing the potential ability of platelet pHi profile to estimate PMI, are needed to validate its potential applicability in forensic scenarios.

Keywords: postmortem interval, cellular biomarkers, platelet pHi; flow cytometry; technique validation

Acknowledgments/Funding

This work received no external funding.

References

1. Secco, L., et al. "Omics" and Postmortem Interval Estimation: A Systematic Review. *Int J Mol Sci.*, **2025**, *26*, 1–25. https://doi.org/10.3390/ijms26031034
2. Woydt, L., et al. Intra-individual alterations of serum markers routinely used in forensic pathology depending on increasing post-mortem interval. *Sci Rep*. **2018**, *8*, 1–12. https://doi.org/10.1038/s41598-018-31252-5
3. Lequerica, J. L. et al. A halocin acting on Na+/H+ exchanger of Haloarchaea as a new type of inhibitor in NHE of mammals. *J Physiol Biochem*. **2006**, *62*, 253–262. https://doi.org/10.1007/BF03165754