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Linking cancer therapy to cardiac injury: Anthracycline Toxicity in Human Heart Cells

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Abstract

Background: Cancer patients are at increased risk of developing cardiotoxicity, largely due to the use of potent anticancer therapies that can damage cardiac tissue [1,2]. Although anthracyclines are widely used and highly effective chemotherapeutic agents, their cardiotoxic effects are frequent and may ultimately progress to heart failure. **Objective:** This study aimed to compare the cytotoxic profiles of two anthracyclines (doxorubicin and daunorubicin) in the human cardiomyocyte cell line AC16 and to investigate whether caspase inhibition can attenuate or reverse drug-induced toxicity. **Methods:** AC16 cells were seeded and maintained in medium supplemented with 12.5% foetal bovine serum and subsequently differentiated for 24 h using 2% horse serum [3]. Differentiated cardiomyocytes were then exposed to doxorubicin and daunorubicin at concentrations ranging from 0.05 to 20 µM. Cytotoxicity was assessed at 24 h and 48 h using the neutral red (NR) uptake and MTT reduction assays. Morphological changes were evaluated by phase-contrast microscopy. To examine the potential prevention of cytotoxicity, a caspase-9 inhibitor was applied to cells treated with 1 and 2.5 µM of each anthracycline. **Results:** Both anthracyclines induced time- and concentration-dependent cytotoxicity, with effects being more pronounced in the MTT reduction assay. At 48 h, the greatest cytotoxic effects were observed with 2.5 µM doxorubicin and with 2.5, 10, and 20 µM daunorubicin in both assays. In contrast, 0.05 µM doxorubicin and 0.1 µM daunorubicin did not produce detectable toxicity at either time point in any of the assays performed. Cellular density and morphology were altered at concentrations ranging from 2.5 to 20 µM after 24 h of exposure and from 1 to 20 µM after 48 h, indicating progressive structural damage over time and with increasing concentrations, with daunorubicin inducing earlier and more pronounced morphological alterations. Treatment with a caspase-9 inhibitor did not attenuate the cytotoxic effects induced by either anthracycline. **Conclusions:** These findings indicate that both doxorubicin and daunorubicin exert cytotoxic effects in AC16 cardiomyocytes, although daunorubicin appears to induce a more pronounced toxic response under the tested conditions.

Keywords: doxorubicin; daunorubicin; AC16 cells; cardio-oncology

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References

1. Reis-Mendes, A. et al. Comparative *in vitro* study of the cytotoxic effects of doxorubicin's main metabolites on cardiac AC16 cells versus the parent drug. *Cardiovasc Toxicol* **2024**, *24*, 266-279, doi:10.1007/s12012-024-09829-6.
2. Dionísio, F. et al. Cardiotoxicity of cyclophosphamide's metabolites: An *in vitro* metabolomics approach in AC16 human cardiomyocytes. *Arch Toxicol* **2022**, *96*, 653-671, doi:10.1007/s00204-021-03204-y.
3. Davidson, M.M. et al. Novel cell lines derived from adult human ventricular cardiomyocytes. *J Mol Cell Cardiol* **2005**, *39*, 133-147, doi:10.1016/j.yjmcc.2005.03.003.