Dysregulation of endothelial cell connexin-43 localisation in response to doxorubicin

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Introduction: Anthracyclines, such as doxorubicin, remain an important class of chemotherapeutic agent however their efficacy in treating cancer is limited by a cumulative dose-dependent cardiotoxicity. Whilst most studies have focused on cardiomyocyte impairment, circulating doxorubicin has been shown to impact human microvascular responses to doxorubicin in coronary vessels.1 Studies show increased endothelial cell permeability resulting in increased paracellular permeability due to damage to the integrity of cell-cell junctions.2 Strategies to maintain vessel integrity and prevent endothelial cell dysregulation could represent a novel therapeutic opportunity to limit the toxic effects of doxorubicin. The aim of this project was to assess the impact of doxorubicin upon endothelial gap junction proteins, in particular connexin-43 (Cx43).

Methods: Commercially available Human Coronary Artery Endothelial Cells (HCAEC) were used for this study. Doxorubicin intrinsic fluorescence was detected at 568 nm therefore drug uptake and accumulation in HCAECs was detected using Leica Confocal SP8 microscope using licensed Leica Application Suite X (LAS X) software. Changes in connexin protein expression were detected using Western blotting and with subcellular localisation assessed using indirect immunofluorescence (IF).

Results: Western blotting confirmed abundant expression of Cx43 in HCAECs with indirect fluorescence demonstrating clustering of Cx43 punctae and gap junction plaque formation. Treatment with doxorubicin (0.1–100µM, 24hrs) resulted in a concentration-dependent accumulation of drug in the nucleus. This coincided with notable subcellular redistribution of Cx43 from the membrane to cytosol, indicative of Cx43 internalisation and decreased endothelial Cx43 expression.

Conclusions: Doxorubicin caused internalisation of endothelial Cx43 and altered the total cellular expression levels of Cx43. The mechanism of Cx43 cellular redistribution and loss remains unknown, however the wider impact of these effects upon healthy cell-cell communication and myoendothelial gap junction activity could be a major contributing factor in doxorubicin cardiotoxicity. Whilst the actions of doxorubicin could be causing Cx43 downregulation through protein degradation, recent evidence suggests that Cx43 can be secreted in extracellular vesicles (EVs).3 Further investigation into the mechanism of aberrant Cx43 expression and potential doxorubicin-dependent Cx43 secretion through EVs will form the basis of future study.

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