EFFECT OF CONTRAST MEDIA ON INTRACELLULAR REACTIVE OXYGEN SPECIES IN ENDOTHELIAL CELLS

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INTRODUCTION

Oxidative stress through generation of reactive oxygen species (ROS) from direct contact with medical imaging contrast media (CM) has been proposed as a likely influencer of contrast induced nephropathy (CIN), a severe, undesirable consequence following angiography that accounts for ~12% of all hospital-acquired acute renal injury. However, results from the use of CM in this setting remain inconclusive. By employing the use of commercially available ROS probes, we tested the hypothesis that CM induces ROS generation exclusively in the mitochondria of cultured vascular endothelial cells, with important consequences for renal vascular perfusion.

MATERIALS AND METHODS

A cell model using EA.hy926 cells was designed to mimic CM delivery *in vivo* during cardiac angiography. The EA.hy926 cell line was selected because endothelial cells are amongst the first cells to come into contact with CM during and after infusion. Confluent EA.hy926 cells were split inro 6 well plates and allowed to grow for 24 hours before being treated with light-exposed CM. To mimic CM infusion and to reflect the rate at which kidneys clear CM from the blood, the cells were initially treated with 100% CM, which was rapidly diluted down to 2% using fresh medium and further diluted by 50% every 2 hours, to reach a final concentration of 0.125%, illustrated in figure 1. Treated cells were left to culture overnight, ROS probe was added, and images captured approximately 24 hours post CM treatment.

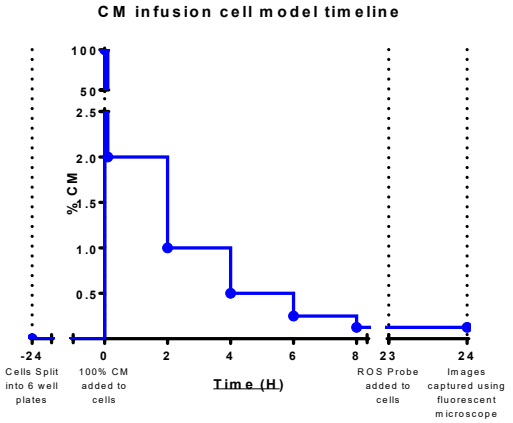

Figure 1 Timeline depicting the *in vitro* CM delivery method developed to mimic CM infusion during cardiac angiography.

Dihydroethdium (DHE) is a fluorescent probe for the detection of ROS generation and was used to measure ROS directly in the cytosol of live cells. DHE is localised to the cytosol and is oxidized by ROS. Once oxidized, it can enter the nucleus, intercalate within DNA, and emit bright red fluorescence.

MitoSOX™ Red is a mitochondria specific ROS probe and was used to detect ROS in the mitochondria of live EA.hy926 cells. MitoSOX™ Red is localised to the mitochondria and is rapidly oxidized by ROS, producing red fluorescence.

RESULTS

DHE

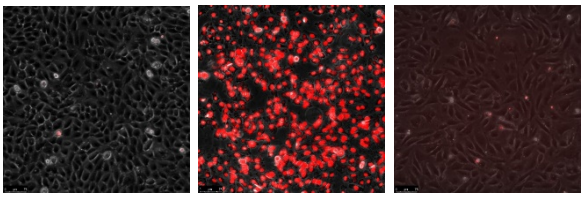

Figure 2 Microscope images showing fluorescence in control cells (left), positive control cells (centre) and CM-treated cells (right). Images were taken at x 40 magnification approximately 24 hours post CM treatment. The scale bar shown in the bottom left corner of each image signifies 75 µm. Bright red fluorescence indicates ROS generation within the cytosol.

MitoSOX™ Red

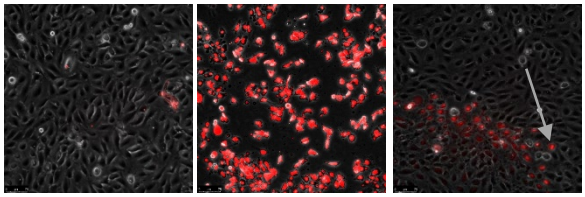

Figure 3 Microscope images showing fluorescence in control cells (left), positive control cells (centre) and CM-treated cells (right). Images were taken at x 40 magnification approximately 24 hours post CM treatment. The scale bar shown in the bottom left corner of each image signifies 75 µm. Bright red fluorescence indicates ROS generation within the mitochondria. An example of a positive CM-treated cell exhibiting bright red fluorescence is represented by the large arrow.

DISCUSSION

These findings suggest that medical imaging CM does not influence ROS production in the cytosol of EA.hy926 cells. Early qualitative findings suggest the potential for increased ROS generation from mitochondria in these cells, but further investigation is required to validate and quantify these results, as well as to determine how the effect is mediated by CM.