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Development and characterisation of a human ex-vivo model of aneurysm

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Introduction: Aortic aneurysm rupture is a cause of premature mortality worldwide, accounting for 2% of all deaths in the UK. Novel treatments to prevent rupture, or biomarkers to identify vulnerability to aneurysm rupture, would therefore represent major scientific breakthroughs. Therefore, mouse models of aneurysm formation have been developed which rely upon angiotensin (Ang-II) or calcium chloride (CaCl₂) as inducers of aortic dilatation. However at least 15% of animals suffer from aortic dissections and subsequent sudden death. Considering this high attrition rate, and a drive to replace, refine and reduce (the 3Rs) the number of animals used in scientific research, there is a need to seek suitable alternatives to study aneurysm pathogenesis, and the testing of potential new therapeutics. To mimic current in vivo animal models, a suitable replacement should harbour similar cellular and morphological features such as vessel dilatation, elastin loss and VSMC apoptosis.

Purpose: The aim of this study is to develop and characterise a potential reproducible ex vivo human model of aneurysm to replace mouse models of aneurysm development, progression and rupture.

Methods: Human umbilical cords were obtained and stored at 4°C immediately after delivery, arteries were isolated from human umbilical cords, placed within a bioreactor and exposed to laminar flow (6.5 dynes/cm²) for 72 hours with and without 5µM Ang-II (n=5), or with and without 5mM CaCl₂ (n=6). Furthermore, we investigated whether introduction of tissue inhibitors of metalloproteinases 3 (TIMP-3) into our Ang II model prevents aneurysm formation, as has been shown in the associated mouse model through perturbation of elastinolytic matrix metalloproteinase activity.

Results: Ex vivo, Ang II stimulation (5µM) for 72 hours significantly reduced umbilical artery medial thickness (37%;p<0.05;n=5) and decreased medial elastin content (47%;p<0.05;n=5), compared to untreated control arteries. Furthermore a significant increase in total vessel area (60%;p<0.05;n=5) as a consequence of vessel dilatation and a significant decrease in the number of medial cells per cross-section (34%;p<0.05;n=5) was also observed after Ang II stimulation (Figure 1). In agreement with published mouse data, addition of recombinant TIMP-3 (5nM) significantly reduced Ang II-induced vessel dilatation (44%;p<0.05;n=6). Similarly, pre-treatment of umbilical artery with CaCl₂ for 15 minutes significantly reduced medial thickness (44%;p<0.05;n=6) and medial elastin content (33%;p<0.01;n=6), although vessel area was unchanged. Conclusion: We demonstrate that our novel ex vivo model with Ang-II infusion or CaCl₂ application of human umbilical cord artery, induced morphological and compositional changes associated with aneurysm formation, which can be reversed by exogenous TIMP-3. Collectively, these findings support the use of this model for aneurysm studies and supplants the need for ethically challenging animal experiments.
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Conclusion: We demonstrate that our novel ex vivo model with Ang-II infusion or CaCl2 application of human umbilical cord artery, induced morphological and compositional changes associated with aneurysm formation, which can be reversed by exogenous TIMP-3. Collectively, these findings support the use of this model for aneurysm studies and supplants the need for ethically challenging animal experiments.

Figure 1. A Total vessel area as an indicator of vessel dilatation was measured in EVG stained sections and percent change in Ang-II compared to control flow for 72 hours is presented (37%;p<0.05; n=5)

B Medial thickness was measured in EVG stained sections and percent change in Ang-II compared to control flow for 72 hours is presented (37%;p<0.05; n=5)

C Elastin content was measured in EVG stained sections and percent change in Ang-II compared to control flow for 72 hours is presented (60%;p<0.05; n=5)

D Medial cell density was assessed in ten x20 magnification fields of H&E stained sections from umbilical cord arteries exposed to flow for 72 hours with and without Ang-II (34%;p<0.05; n=5)