ANGIOPOIETIN 1 SIGNALLING, A MARKER OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH CORONARY ARTERY DISEASE

Harprit Singh, Nick Brindle. *University of Leicester, Cardiovascular Sciences, Robert Kilpatrick Building, PO Box 65, Leicester. LE2 7LX, UK*

Background: Angiopoietin1 (Ang1) is a ligand crucial for vascular protection, maintains normal endothelial function and helps to preserve healthy vessels. Ang1 signals through specific Tie2 receptor proteins that are present on the surface of endothelial cells. Binding of Ang1 activates Tie2 receptors allowing various signalling pathways to be activated within the cell. Some of the important pathways include phosphatidylinositol-3-kinase (PI-3K)/AKT and Erk1/2 have been implicated in the po-survival and protective activities of Ang1. We have shown that a variety of pathophysiological effectors including elevated glucose and free fatty acids significantly impair this protective Ang1/Tie2 signalling in primary endothelial cell cultures (Singh *et al.*, 2010). From this we hypothesised that endothelial cells from patients with vascular disease have impaired Angiopoietin 1 protective signalling.

Methods: To investigate this we optimized conditions for the isolation of endothelial cells from distal ends of catheters used on patients during coronary catheterization angiography. Approximately, 5-8 cm of the ends of the catheters were removed after coronary angiography procedure and placed in endothelial dissociation buffer (EDB) for cell isolation. Cells were separated from EDB by centrifugation and cultured in endothelial specific growth medium. These cells showed typical staining for von WIllebrand Factor (vWF), a marker of endothelial cells. Using this technique, we isolated human endothelial cells (HEC) from 13 patients that were referred to catheter laboratory for coronary angiography. Patients that displayed normal angiograms were referred as control patients (N=4). Angiograms from patients that showed coronary arteries with $\geq$ 50% reduction in diameter in one or more areas were characterized as Coronary Artery Disease (CAD) patients (N=9). HEC isolated from the tips of used coronary catheters on these patients were plated in culture immunofluorescent dishes and left to adhere for 2 hours in the presence of endothelial media. Cells were then stimulated with or without 200ng/ml Ang1, fixed and subjected to dual immunofluorescence staining using specific antibodies targeting Phospho-Tie2 and Phospho-Erk1/2. Cells were visualized under epifluorescence microscope and quantification of relative Tie2 and Erk1/2 phosphorylation between Control and CAD were performed by comparing mean intensity of at least 15 cells per individual samples treated with Ang1. Statistical analysis was performed using unpaired t-test (*p<0.05*).

Results: Patients with CAD showed significant decrease in Ang1-mediated Tie2 (52 ± 0.47% *p<0.05* vs. Control) and Erk 1/2 (57 ± 0.36% *p<0.05* vs. Control) phosphorylation.

Conclusion: These initial experiments have confirmed this technique can be utilized to examine Ang1 signalling directly in patient-derived endothelial cells and suggests that Ang1 signalling is defective in these cells from CAD patients. This makes the Angiopoietin 1 signalling pathway a possible target for pharmacological agents for the treatment of endothelial dysfunction in patients with vascular disease.

Singh *et al* (2010) *Microvascular Research* **79**, 121-127