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Cyclo-oxygenase Immunoreactivity in Human Aortic Endothelial Cells Cultured under Static and Shear Stress Conditions

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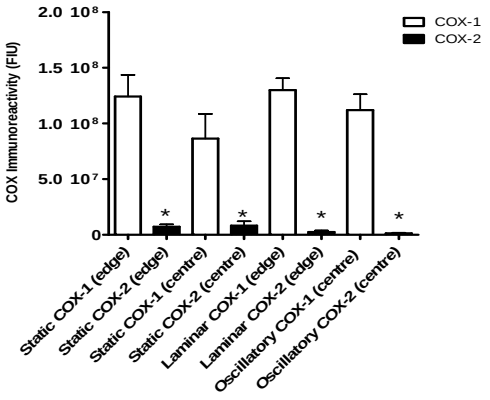
Endothelial cells produce the cardioprotective and anti-thrombotic hormone prostacyclin, whilst platelets produce the pro-thrombotic hormone thromboxane A_2, through the metabolism of arachidonic acid. Prostacyclin and thromboxane A_2 are both formed from prostaglandin H_2 via the enzyme cyclo-oxygenase (COX). At the site of inflammation COX also forms pro-inflammatory mediators including PGE_2. COX exists in two isoforms, COX-1 and COX-2 and is the enzyme target for non-steroidal anti-inflammatory drugs (NSAIDs). It has been suggested that the cardiovascular side effects associated with COX-2 selective NSAIDs are due to the selective inhibition of endothelial prostacyclin over platelet thromboxane A_2 synthesis. Detecting COX-2 in the endothelium has proved difficult although its expression can be induced when endothelial cells are exposed to shear stress for short time periods. However, in order to mimic *in vivo* conditions it is important to determine the levels of COX-1 and COX-2 in endothelial cells exposed to shear stress for prolonged periods.

Using a novel model in which cells are grown on Transwell plates placed on an orbital shaker both a laminar (edge of the well) and oscillatory shear stress (centre of the well) environment can be generated. We were able to culture human aortic endothelial cells under shear stress in this way for up to 7 days. We then used immunohistochemistry and en face confocal microscopy to assess COX-1 and COX-2 immunoreactivity.

We saw no elevation in COX-1 or COX-2 after application of laminar shear or oscillatory shear. COX-1 immunoreactivity was significantly greater than COX-2 immunoreactivity under all conditions studied. This confirms the results of our earlier study in porcine cells (Potter et al; 2009, PA2 online 043P) and is consistent with the idea that COX-1 predominates in vascular endothelial cells even when exposed to chronic shear stress.



COX immunoreactivity in human aortic endothelial cells cultured for 7 days under static or shear stress conditions. Data is mean +/- S.E.M for n=4-6. Data was analysed using One-way ANOVA followed by Bonferroni post test;

* denotes p=<0.05 compared to COX-1 immunoreactivity under all conditions