Relative expression of cyclo-oxygenase COX-1 and COX-2 in porcine endothelium subjected to chronic shear stress

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Endothelial cells are enriched with cyclo-oxygenase (COX) which, together with prostacyclin synthase, catalyses the conversion of arachidonic acid to the anti-thrombotic hormone prostacyclin. Generally, COX-1 is expressed constitutively whilst COX-2 is induced at sites of inflammation. COX-1 predominates over COX-2 in endothelial cells cultured under static conditions. However, it is unclear which isoform predominates in endothelial cells cultured under shear stress, as is normally experienced by endothelial cells in vivo. Because experiments employing short bursts of shear stress can provide misleading results, since these can be perceived by cells as an inflammatory insult, we have studied COX-1 and COX-2 expression in cells cultured under shear stress for prolonged periods.

Porcine aortic endothelial cells were grown to confluence in 6-well Transwell™ plates. Cells were then cultured for up to 7 days under either static conditions or under shear stress produced by placing the plates on a rotating platform. In this model cells at the centre of the well experience ‘low shear’ of 2 dynes/cm² whilst those towards the outer edge experience ‘high shear’ of approximately 4 dynes/cm². COX-1 and COX-2 immunoreactivity within cells was then imaged using enface confocal microscopy and quantified using Volocity 5 software. In separate experiments porcine aortic endothelial cells grown under static conditions were stimulated to express COX-2 by incubation with LPS (0.1µg/well) for 24h.

Endothelial cells expressed both COX-1 and COX-2 with COX-1 being the dominant isoform under both static conditions and after 7 days of shear stress. Endothelial cells could readily express COX-2, as seen following exposure to LPS. These observations are consistent with the idea that COX-1 predominates in vascular endothelial cells even when exposed to chronic shear stress.