Analysis of regional distribution of COX-isoforms in endothelial cells of the aortic arch of the mouse reveals a predominance of COX-1 in healthy blood vessels

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Endothelial cells contain cyclooxygenase (COX) which, together with prostacyclin (PGI_2) synthase, catalyses the formation of the cardioprotective and anti-thrombotic hormone, PGI_2, in blood vessels. COX is expressed in two isoforms, COX-1 and COX-2. Inhibition of COX-2 underlies the therapeutic effects of non-steroidal anti-inflammatory drugs. Whilst evidence suggests that COX-1 predominates over COX-2 in endothelial cells in culture, the isoform present in endothelial cells *in vivo* is still the subject of investigation. Endothelial cells have heterogeneous phenotypes including those associated with different types of hemodynamic flow patterns. In the ascending aortic arch there are two well defined areas on the luminal surface at which endothelial cells display flow-responsive phenotypes. Endothelial cells in the greater curvature of the aortic arch, which experience predominately unidirectional laminar flow are uniformly spaced, with an elongated shape orientated in line with the direction of the blood flow and are protected from atherosclerosis (athero-protected area). In contrast, endothelial cells in the lesser curvature, which experience oscillatory flow, are irregular in shape, randomly orientated, more sensitive to inflammation and show an increased susceptibility to atherosclerotic plaque formation (athero-prone area). Here, we have investigated by immunohistochemistry the distribution of COX-1 and COX-2 in endothelial cells of the athero-protected and athero-prone regions in aortic arches from C57Bl6 mice, using *en face* confocal microscopy (1). Immunoreactivity was quantified as total florescence intensity (FI) per picture after subtraction of background. We also determined COX activity in the endothelial layer of aortic arch sections by analyzing formation of PGI_2 (measured as 6-keto$PGF_{1\alpha}$ by RIA) after incubation with A23187 ($5x10^{-5}$M, 30min); comparisons were made between tissue of wild type (wt; C57Bl6) and COX-$1^{-/-}$ mice.

Results: COX-1 was highly expressed in endothelial cells of all regions of the aortic arch, and tended to be higher in the athero-prone area ($2.77x10^8$ ± $0.39x10^8$ FI, n=6) than the athero-protected area ($1.92x10^8$ ± $0.48x10^8$ FI, n=6). COX-2 was undetectable in endothelial cells of the athero-protected area ($0.08x10^8$ ± $0.17x10^8$ FI, n=6), but low levels were detected in endothelial cells of the athero-prone area ($0.43x10^8$ ± $0.11x10^8$ FI, n=6). Aortic arch sections from wt (n=7) and COX-$2^{-/-}$ (n=6) mice produced more than ten times more PGI_2 (wt, 19.98 ± 0.80; COX-$2^{-/-}$, 15.24±1.21 ng/ml) than aortic arches from COX-$1^{-/-}$ mice (1.46±0.68 ng/ml, n=7).

Conclusion: This data show that COX-1 predominates in endothelial cells of the aortic arch and is responsible for the majority of PGI_2 production in normal tissue. The consequences of enriched COX-1 and detectable COX-2 expression in the athero-prone area of the aortic arch remain the subject of investigation.

1.Hajra, L. et al., 2000, *PNAS* 97:9052-9057.