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Deletion of COX-2 augments atherosclerosis and vascular inflammation in ApoE$^{-/-}$ mice, independently of local prostacyclin production

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Chronic use of selective COX-2 inhibitors (e.g. rofecoxib) or non-selective COX-1/COX-2 inhibitors (e.g. ibuprofen) is associated with an increase in atherothrombotic events. This is widely suggested to be a result of loss of COX-2-dependent production of protective prostacyclin in the vessel wall[1] (Grosser *et al.* 2006). We have recently demonstrated, however, that COX-1 rather than COX-2 drives prostacyclin synthesis in healthy animals and human cells[2]. Here we have extended these observations to determine if atherosclerotic disease can drive vascular COX-2-expression, and what consequences this may have on atherosclerotic lesion development.

ApoE$^{-/-}$ and ApoE$^{-/-}$/COX-2$^{-/-}$ mice (12 weeks old, male+female) were fed an atherogenic diet (1.25% cholesterol) for 10 weeks to induce lesion formation. COX-1 and COX-2 expression were determined by en face immunofluorescence in the aortic arch. Aortic prostacyclin production was measured by incubating segments of aortic arch with calcium ionophore A23187 (50μM in 0.1% DMSO/DMEM) for 30mins, and measurement of the prostacyclin breakdown product 6-keto-PGF1α by ELISA. Aortic lipid accumulation was determined by en face sudan IV staining of the aortic tree, and atherosclerotic lesion burden by optical projection tomography (OPT) imaging of the aortic arch[3]. Circulating cytokine levels were determined in plasma by ELISA and cholesterol levels by a commercial veterinary biochemistry service (IDEXX Labs). Data were analysed by unpaired t-test and are expressed as mean ± SEM.

The aortic arch of fat-fed ApoE$^{-/-}$ contained abundant COX-1 immunoreactivity with only sparse, sporadic COX-2 immunoreactivity, despite the presence of atherosclerotic lesions. In agreement, deletion of COX-2 had no effect on the ability of aortic arch tissue to generate prostacyclin (ApoE$^{-/-}$: 11.7±1.0ng/ml; ApoE$^{-/-}$/COX-2$^{-/-}$: 13.2±2.3ng/ml; p=0.64; n=5) or plasma levels of prostacyclin (ApoE$^{-/-}$: 0.42±0.11ng/ml; ApoE$^{-/-}$/COX-2$^{-/-}$: 0.44±0.07ng/ml; p=0.90; n=10). Despite this, ApoE$^{-/-}$ mice lacking COX-2 demonstrated increased lipid deposition in the aortic arch (ApoE$^{-/-}$: 2.6±0.4mm^2; ApoE$^{-/-}$/COX-2$^{-/-}$: 5.1±0.7mm^2; p=0.003; n=10), and increased atherosclerotic lesion volume in the aortic arch, carotid, subclavian and brachiocephalic arteries (e.g. in the brachiocephalic artery, ApoE$^{-/-}$: 0.10±0.01mm^3; ApoE$^{-/-}$/COX-2$^{-/-}$: 0.17±0.01; p=0.003; n=10). This was accompanied by increased circulating levels of cholesterol (ApoE$^{-/-}$: 23±1mM; ApoE$^{-/-}$/COX-2$^{-/-}$: 30±2mM; p=0.005; n=10), IL-2 (ApoE$^{-/-}$: 2.9±0.4pg/ml; ApoE$^{-/-}$/COX-2$^{-/-}$: 4.3±0.5pg/ml; p=0.03; n=25), IL-12 (ApoE$^{-/-}$: 0.8±0.1ng/ml; ApoE$^{-/-}$/COX-2$^{-/-}$: 1.2±0.2; p=0.02; n=25) and the mouse IL-8 homolog, KC (ApoE$^{-/-}$: 57±7pg/ml; ApoE$^{-/-}$/COX-2$^{-/-}$: 121±20pg/ml; p=0.003; n=25).

Thus, COX-1 is the dominant isoform mediating prostacyclin production in atherosclerotic mouse vessels with little contribution of COX-2. Nonetheless, deletion of COX-2 increases atherosclerosis and this is associated with increased circulating lipid and cytokine levels. Although the mechanism by which loss of COX-2 produces these changes remains to be determined, these data indicate that COX-2, at a distant site, can produce profound changes in lesion formation in the vascular wall. If a similar process occurs in man, this may provide some explanation for the association between COX-2 inhibition and atherothrombotic events.

1: Grosser et al. *J Clin Invest*. 2006. **116**(1):4-15.

2: Kirkby NS et al. *Proc Nat Acad Sci*. 2012. In press.

3: Kirkby NS et al. *PLoS One*. 2011. **6**(2):e16906.