Functional investigation of the role of cyclo-oxygenase inhibitors on platelet function in vivo in the mouse

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Background: Cyclo-oxygenase (COX) enzymes produce the pro-thrombotic mediator thromboxane A2 and the antithrombotic mediator prostacyclin, and have been a target for thrombotic modulation for many years. However, COX-2 selective drugs are contraindicated in patients with underlying cardiovascular problems, due to evidence that they increase the risk of thrombotic events. The underlying cause of this increased risk is yet to be fully established but has been suggested to involve the inhibition of prostacyclin from COX-2 in the vascular endothelium.

Experimental approach: We contrasted the effects of Naproxen (10mg.kg⁻¹, 30mg.kg⁻¹ and 100mg.kg⁻¹), a non selective COX-1 and COX-2 inhibitor, and nimesulide (1mg.kg⁻¹ and 10mg.kg⁻¹), a selective COX-2 inhibitor, by monitoring the effects of these compounds on the in vivo aggregation response of radiolabelled platelets to collagen in real-time in the anaesthetised mouse. Platelets were isolated from a donor C57BL6/J mouse, labelled with ¹¹¹In and injected into an anaesthetised recipient mouse (Urethane-25%w/v at 10μl/g i.p.). The response of the circulating radiolabelled platelets to collagen, before and after the administration of experimental drugs, was then assessed in real-time by measuring the accumulation of radioactivity within the pulmonary vasculature using an external scintillation probe.

Results: Naproxen produced an anti-thrombotic effect shown as a decrease in the platelet aggregation response in vivo (%Δ in maximum response to collagen from pre drug response: Saline 16.5±8.6%, 10mg.kg⁻¹ -15.9±8.2% (P<0.01), 30mg.kg⁻¹ -22.8±3.3% (P<0.01), 100mg.kg⁻¹ -37.5±2.2% (P<0.001)), whereas nimesulide did not affect the thrombotic response (%Δ in maximum response to collagen from pre drug response: Saline 5.3±3.8%, 1mg.kg⁻¹ 4.7±5.1% (ns), 10mg.kg⁻¹ -5.8±9.6% (ns)). The haemodynamic consequences of the thrombotic response were also measured by invasive cannulation of the left ventricle via the carotid artery with a 1.4Fr Millar pressure-volume catheter, and supported the conclusion that Naproxen has anti-thrombotic effects, measured as a lower impairment of contractile function (%Δ in dP/dtmax: Saline -34.7±1.2%, 10mg.kg⁻¹ -12.5±2.0% (P<0.01), 30mg.kg⁻¹ -19.7±2.8% (P<0.05), 100mg.kg⁻¹ -13.6±0.8% (P<0.01)) during thrombotic stimulation. Data was analysed using one way ANOVA with Benferroni post-analysis against a time matched vehicle control.

Conclusions and further work: The data presented opposes the suggestion that the pro-thrombotic effects of COX-2 selective drugs are due to inhibition of constitutive COX-2 production of prostacyclin in the endothelium and subsequent enhancement of platelet responsiveness. This work provides a platform for investigating the consequences and mechanisms of COX-2 inhibition upon platelet function and thrombotic risk in models of inflammation or vascular dysfunction, including atherosclerosis, where the role of COX-2 may be altered.