

**Blood pressure effects of the COX-2 inhibitor parecoxib are associated with elevated levels of the endogenous eNOS inhibitors L-NG-monomethyl L-arginine (LNMMA) and asymmetric dimethyl L-arginine (ADMA)**

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Inhibition of cyclo-oxygenase (COX)-2 by nonsteroidal anti-inflammatory drugs (NSAIDs), including COX-2 selective inhibitors such as rofecoxib and celecoxib, is associated with cardiovascular side effects. It was previously thought that the mechanism by which COX-2 inhibitors precipitate these side effects was simply explained; that COX-2 drives the vascular production of the cardio-protective hormone, prostacyclin, and that its blockade predisposes to thrombosis and atherosclerosis. However, we have recently shown that vascular prostacyclin is COX-1 driven. We are now left with no clear explanation of how COX-2 inhibitors precipitate cardiovascular side effects. COX-2 is expressed in key anatomical hot spots, including the kidney (Kirkby *et al.*, 2013) where its inhibition or genetic deletion results in renal dysfunction (Harris, 2006). Separate studies have shown that renal dysfunction is associated with a reduction of the enzyme DDAH1, which removes the endogenous inhibitor of NO synthase (NOS), asymmetric dimethyl L-arginine (ADMA) (Arrighi *et al.*, 2010).

Our working hypothesis is that 'cardiovascular side effects of COX-2 inhibitors are mediated (in part) by increases in ADMA and the associated loss of protective eNOS activity'. In other studies we have found that genetic deletion of COX-2 in mice reduces renal DDAH1 activity and increases plasma levels of ADMA (unpublished data). However, global gene deleted mice can carry phenotypic abnormalities not directly associated with the candidate gene. Furthermore, developmental and lifelong loss of COX-2 in genetically modified mice may not translate to what happens with clinically relevant COX-2 inhibitors. Thus the purpose of this study was to investigate the effects of the COX-2 inhibitor, parecoxib (water soluble formulation of valdecoxib), on blood pressure and circulating levels of ADMA. C57BL/6 male mice ( $n \geq 5$ ) were given vehicle (water only) or parecoxib (100mg/kg)  $\pm$  L-arginine (0.1% w/v) for 4 days in their drinking water. Blood pressure was determined following 24 hour telemetry recordings. Total NO (NOx) and arginine/methylarginine plasma concentrations were quantified using the Sievers NO analyser and LC-MS/MS respectively.

Parecoxib significantly increased systolic arterial pressure (control,  $113 \pm 5$  mmHg; parecoxib,  $128 \pm 4$  mmHg;  $p < 0.001$ ) and mean arterial blood pressure (control,  $101 \pm 4$  mmHg; parecoxib,  $112 \pm 6$  mmHg;  $p < 0.05$ ) during the light period. This was correlated with significantly elevated plasma ADMA (control,  $0.41 \pm 0.04$   $\mu$ M; parecoxib,  $0.86 \pm 0.13$   $\mu$ M;  $p < 0.05$ ) and L-NMMA (control,  $0.33 \pm 0.07$   $\mu$ M; parecoxib,  $1.19 \pm 0.26$   $\mu$ M;  $p < 0.05$ ) and reduced L-arginine (control,  $186 \pm 30$   $\mu$ M; parecoxib,  $120 \pm 24$   $\mu$ M;  $p < 0.05$ ). L-arginine supplementation had no significant effect on blood

pressure yet tended to increase plasma NO<sub>x</sub> (parecoxib, 46.1±7.3μM; parecoxib and L-arginine, 68.6±9.6μM) and reduce ADMA (parecoxib, 0.86±0.13μM; parecoxib and L-arginine, 0.59±0.11μM) and L-NMMA (parecoxib, 1.19±0.26μM; parecoxib and L-arginine, 0.54±0.09μM;  $p<0.05$ ).

These observations provide a novel explanation for how COX-2 selective inhibitors cause cardiovascular dysfunction and highlight the possible therapeutic potential of L-arginine supplementation.

Arrigoni F et al. (2010). *Curr Pharm Des* **16**(37): 4089-4102.

Harris RC (2006). *J Cardiovasc Pharmacol* **47 Suppl 1**: S37-42.

Kirkby NS et al. (2013). *PLoS One* **8**(7): e69524.