ACTIVATION OF PROTEASE-ACTIVATED RECEPTOR-2 (PAR-2) INDUCES CARDIOPROTECTION IN ANAESTHETISED MICE

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PAR2 activation has been shown to mediate cardioprotection in isolated rat hearts (Napoli *et al*., 2000). The present study aimed to determine whether the PAR2 agonist, trypsin and the activating peptide (SLIGRL-NH_2, SL-NH_2), could protect the heart from acute myocardial ischaemia and reperfusion injury (MIRI) *in vivo*. To confirm an action through PAR2, studies were performed in wild type (PAR2+/+) and PAR2 knockout (PAR2-/-) mice. The adenosine A_1 receptor agonist, 2-chloro-N^6-cyclopentyl-adenosine (CCPA), was studied as a cardioprotective agent acting via a PAR2 independent mechanism.

PAR2-/- mice were produced by back-crossing 5 times with C57BL/6 mice and the knockout confirmed by genotyping. 14-16 week old PAR2+/+ and PAR2-/- mice (18-34g) were anaesthetised with a combination of ketamine, xylazine and atropine (intraperitoneally) and artificially ventilated with O_2. The left anterior descending coronary artery was ligated to induce 30 min of occlusion followed by 120 min of reperfusion. Infarct size was assessed by Evan's blue-triphenyltetrazolium chloride dual staining. Mice were pre-treated with a bolus dose (0.05 ml/ 25 g) of saline (vehicle control), trypsin (1 U/g), SL-NH_2 (0.3 nmol/g) or CCPA (100 ng/g) intravenously via the tail vein 24-hour prior to surgery. Statistical significance was assessed using one way ANOVA with Dunnett's post-hoc test.

A significant reduction in infarct size (expressed as percentage of the area at risk, AAR) was observed in PAR2+/+ mice pre-treated with trypsin (49±3%; n=11), SL-NH_2 (41±4%; n=10) and CCPA (39±4%; n=10) when compared to the vehicle control (59±2%; n=14). These effects of trypsin and SLIGRL-NH_2 were not seen in PAR2-/- mice (58±5% (trypsin, n=7), 59±2% (SL-NH_2, n=6) vs. 56±4% (saline, n=9); $p>0.05$), although CCPA still reduced infarct size (41±4%, n=8; $p<0.05$). The AAR was similar in hearts from all groups. In untreated mice, PAR2 deletion by itself did not modify infarct size (56±3% vs. 54±5% in PAR2+/+ (n=11) and PAR2-/- (n=12) mice respectively; $p>0.05$). The respective control arterial blood pressures (mmHg) and heart rates (beat per minute) in PAR2+/+ (123±5 and 388±14) and PAR2-/- (132±5 and 407±10) mice, prior to coronary artery occlusion, were not significantly modified by pre-treatment with trypsin, SL-NH_2 or CCPA.

We conclude that exogenously applied trypsin and SL-NH_2 have a cardioprotective action on MIRI in the mouse and this effect is PAR2 mediated as demonstrated by the lack of a response in the PAR2-/- mice. However, infarct size was not modified by deletion of the PAR-2 itself.

Napoli C. *et al*. (2000). *Proc. Natl. Acad. Sci. USA.* **97**: 3678.

S.Y. Lim holds an ORS & Strathclyde University studentship. PAR2 mice and PAR-2 activating peptide are courtesy of Prof. Robin Pelvin.