

Manipulating the innate immune system as a cardioprotective strategy

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Myocardial ischaemia-reperfusion injury (IRI) occurs when blood flow is restored to the myocardium following a period of ischaemia, such as after revascularisation after infarction, or reperfusion after cardioplegia. Despite intense research, strategies to abrogate or minimise IRI are not effective. The mechanisms underlying IRI include damage to mitochondria, generation of ROS, disturbance of ion fluxes (particularly Ca²⁺ overload), and contractile protein dysfunction. Inflammation, *via* activation of the immune system, also plays a large role. The innate immune system is the more “primitive” part of immune defence, which recognises a range of small molecules rather than specific antigens. Part of this recognition is by Toll-like receptors (TLRs), which are expressed in the heart. It has been proposed that during reperfusion activation of TLR4 receptors contributes to IRI, based on the finding that knockdown of TLR4 receptors reduces IRI (Oyama *et al.* 2004).

A proven non-pharmacological method to protect the myocardium from IRI is afforded by ischaemic preconditioning, whereby exposure of the heart to brief transient periods of ischaemia can reduce the damage caused by a subsequent longer period of ischaemia (Murry *et al.*, 1986). However, no dominant single mechanism seems to be involved, and attempts to produce “pharmacological preconditioning”, or cardioprotection, have been unsuccessful. We have therefore examined whether TLR4 receptors are involved in ischaemic preconditioning.

It has been shown that LPS (an agonist of TLR4 receptors) is cardioprotective (Rowland *et al.*, 1997). We hypothesized that this is due to desensitisation of TLR4 receptors, and that similar activation/desensitisation of TLR4 receptors by transient ischaemia may be a mechanism of preconditioning. If so, pharmacological block of TLR4 during preconditioning should abrogate the protective effect. To test this idea, we used amitriptyline, an antagonist at TLR4 receptors, in a preconditioning protocol in isolated rat hearts. Surprisingly, amitriptyline (10 µM) did not affect preconditioning, but was itself cardioprotective. We ascribe this to off-target actions (Lee *et al.*, 2015).

We have therefore recently used more specific compounds, naloxone and naltrexone in the same way. The (+) isomers of these compounds do not interact with opioid receptors, but they are antagonists at the TLR4 receptor (Hutchinson *et al.* 2008). In an isolated mouse heart model of preconditioning, we find that either naltrexone or naloxone (20 µM) did not affect IRI itself, but did impede recovery of LVDP after ischaemic preconditioning if they were present during the preconditioning period.

We conclude that TLR4 receptors may play a role in ischaemic preconditioning.

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