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# COMMENTARY

# Reperfusion and calculated RISKs: pharmacological postconditioning of human myocardium

GF Baxter and DS Burley

*Division of Pharmacology, Welsh School of Pharmacy, Cardiff University, Cardiff, UK*

The last five years have witnessed a remarkable resurgence of interest in myocardial reperfusion injury. Reperfusion is absolutely essential to salvage ischaemic myocardium but experimental and clinical studies show that reperfusion-associated injury may mask the full benefits of prompt reperfusion in acute myocardial infarction. In the current issue of the *British Journal of Pharmacology*, Mudalagiri *et al* demonstrate a protective effect against simulated reperfusion injury using exogenously applied erythropoietin in human isolated myocardium. Crucially, the benefits of erythropoietin were observed when it was administered specifically during re-oxygenation. The demonstration that the protective effects of the cytokine were dependent on PI3-kinase/Akt and ERK1/2 activation provides compelling evidence that reperfusion injury salvage kinases (RISKs) are key survival mechanisms in human myocardium, as they are in experimental animal species. Although erythropoietin may be only one of several potential pharmacological approaches in human patients, this study establishes the important proof-of-principle that activation of RISKs is protective in human myocardium and could be a promising therapeutic target in acute myocardial infarction.
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In acute myocardial infarction, unrelieved ischaemia results in extensive tissue necrosis and it is likely that multiple signalling pathways regulate the balance between cells succumbing to or surviving the ischaemic insult. The success of reperfusion/revascularization therapy in limiting infarct size and in influencing long-term prognosis is, in very simple terms, dependent on the speed with which it is applied: the axiom 'time is muscle, and muscle is life' nicely encapsulates this therapeutic principle. However, despite the unquestioned necessity and success of reperfusion in preventing ischaemic necrosis, there is a considerable body of experimental evidence that reperfusion is associated with activation of lethal signals that culminate in further necrosis (or apoptosis) of viable but vulnerable myocardium that has survived the ischaemic period. Indeed, recent developments suggest that events in early reperfusion contribute to a far greater extent than it was previously supposed. This prompts a re-assessment of reperfusion injury mechanisms and opens up the opportunity for targeting the early reperfusion period therapeutically ('postconditioning') to maximize the benefits of reperfusion/revascularization in acute myocardial infarction. A study by Mudalagiri *et al*. (2007) reported in the current issue of the *British Journal of Pharmacology* provides proof-of-principle that pharmacological postconditioning, in this case with erythropoietin, may afford an approach to targeting reperfusion injury in human myocardium.

The study by Mudalagiri *et al*. is based on studies in animal models that have identified the activation of the classical survival (antiapoptotic) kinases as a pivotal feature of diverse forms of experimental infarct-limiting manoeuvres. Several lines of evidence support the notion that phosphatidylinositol-3 kinase/Akt (PI-3 kinase/Akt) and p42/p44 MAP kinases (ERK 1/2) promote cell survival during reperfusion (Hausenloy and Yellon, 2007). Other kinases may also play a prosurvival role at reperfusion, notably cyclic GMP-dependent protein kinase (PKG) (Piper *et al*., 2004; Burley *et al*., 2007). Yellon and his co-workers have coined the term 'reperfusion injury salvage kinases' or 'RISKs', a useful conceptual shorthand for this cell survival programme and a catchy acronym. Injury-limiting interventions that have been identified to exert cardioprotective actions through RISK activation include insulin and other peptide growth factors, statins, cardiac peptide hormones, erythropoietin, flurane anaesthetics, ischaemic preconditioning (applied before the onset of ischaemia) and ischaemic postconditioning (applied at the onset of reperfusion). The RISK pathways have multiple downstream mechanisms that could contribute to cytoprotection, including activation of NOS, inhibition of the mitochondrial permeability transition pore

Correspondence: Professor GF Baxter, Division of Pharmacology, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3NB, UK.
E-mail: baxtergf@cardiff.ac.uk
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(mPTP), upregulation of antiapoptotic members and/or inhibition of proapoptotic members of the Bcl2 protein family, regulation of sarcoplasmic reticulum $Ca^{2+}$ uptake and release and inhibition of glycogen synthase kinase-3β. It is not clear how these distal mechanisms are related to one another, but inhibition of mPTP opening seems to be a critical convergence point. After ischaemia of sufficient duration, the conditions of early reperfusion promote the opening of mPTP and this almost certainly represents a final trigger for cell death. Although the idea has taken a long time to gain general acceptance (largely because throughout the 1990s our major focus of interest was preconditioning and signalling events during ischaemia), there is now a consensus of opinion that inhibition of mPTP opening during reperfusion, probably as a result of activation of the RISK pathway, is a primary common effector mechanism for many, perhaps most, experimental infarct-limiting manoeuvres.

The model system employed by Mudalagiri *et al*. was human isolated atrial trabeculae. The diminution of electrically stimulated isometric developed force during simulated ischaemia and the recovery of contractility measured during re-oxygenation follow a very characteristic pattern, similar to that seen in isolated perfused hearts subjected to global ischaemia and reperfusion. The key question addressed was the ability of erythropoietin to limit 'reperfusion' injury when administered specifically during the re-oxygenation period, mimicking the putative clinical scenario in which a cardioprotective agent would be given at the onset of reperfusion as a pharmacological post conditioning agent.

The ability of erythropoietin to enhance recovery of contractile function was prevented by pharmacological inhibitors of either PI3-kinase activation (LY294002) or ERK1/2 activation (UO126). This finding corroborates, and extends, previous work by the same group in rat myocardium showing that administration of erythropoietin at the onset of reperfusion limited infarct size in a PI3-kinase and ERK1/2 dependent manner (Bullard *et al*., 2005). The work also complements nicely a recent study in human isolated atrial myocardium showing that hypoxic postconditioning (that is, brief periods of intermittent hypoxia applied at the onset of re-oxygenation) enhances recovery of function through these RISK pathways (Sivaraman *et al*., 2007). Thus, the current study provides evidence that human myocardium can be pharmacologically postconditioned against reperfusion/re-oxygenation injury by recruitment of the RISK pathways. A technical limitation to interpretation of the current study, however, is the application of erythropoietin throughout the full re-oxygenation period. In the post conditioning paradigm in which we are now working, transient activation of RISKs during the early period of reperfusion is necessary and sufficient to induce protection. It is conceivable that an inotropic effect of erythropoietin on stunned cells could account for the improvement in contractile recovery seen throughout the re-oxygenation period; to discount this possibility it would have been desirable to remove erythropoietin from the re-oxygenation buffer after 30 min or so and observe if the recovery of function was maintained.

Although much of the post conditioning literature, including the current report, has focused on the critical role of RISKs, erythropoietin is known to influence multiple signalling pathways and the contribution of kinases other than RISKs might be relevant to the current picture. Other work suggests roles of phosphorylation and activation of JAK1/2, STAT3 and STAT5A2, PI3-kinase/Akt, PKCε, Raf, p38 MAPK, and MEK1/2 upstream of ERK1/2 in the cardioprotective effect of erythropoietin given prior to the onset of ischaemia in rabbit isolated heart (Rafiee *et al*., 2005). Although activation of NOS does not appear to play a role, ATP-sensitive $K^+$ ($K_{ATP}$) channel opening appears to be relevant to protection afforded by pre-ischaemic erythropoietin (Shi *et al*., 2004). Arguably, as a pleiotropic cytokine activating multiple signalling pathways (and hence with the potential to exert multiple unwanted effects), erythropoietin may not be the ideal theoretical choice for an adjunct to clinical reperfusion.

In his influential essay on the process of scientific discovery, Kuhn (1962) referred to scientific crises and revolutions, resulting in paradigm shifts. In the field of ischaemia–reperfusion injury and cardioprotection research, it is not an overstatement to say that we are witnessing currently all the features of such a paradigm shift. What is truly exciting is that the new reperfusion injury paradigm that has emerged after the years of 'puzzle-solving' in the previous preconditioning paradigm has the potential to translate very rapidly to a major clinical advance. Erythropoietin seems to have caught the imagination of cardiologists (Lipsic *et al*., 2006), but whether it will be the adjunctive therapy of choice in acute myocardial infarction remains to be seen. However, the demonstration that human myocardium displays the key features of the RISK programme seen in animal models is an encouraging and significant step in the preparation for clinical studies. As with all clinical studies, the devil will be in the detail of trial design, and no trial is without financial risk. But we have a clearer picture now than ever before of what settings and what agents will maximize the chances of success. The clinical and economic benefits of a successful pharmacological post conditioning agent could be immense. A question hanging in the air is: will the industry take a calculated risk on RISKs?

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