

# Cardiac preconditioning for ischaemia: lost in translation

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Coronary heart disease (CHD) is the leading cause of death worldwide. The development of novel treatment strategies for protecting the myocardium against the detrimental effects of acute ischaemia-reperfusion injury, termed cardioprotection, and for improving clinical outcomes in patients with CHD requires the use of appropriate animal disease models. The concept of cardioprotection was first conceived in the late 1960s and has evolved to include the endogenous cardioprotective phenomenon of ischaemic conditioning, a concept in which the heart can be protected from an episode of acute lethal ischaemia-reperfusion injury by applying brief non-lethal episodes of ischaemia and reperfusion either to the heart itself or to an organ or tissue that is remote from the heart. The brief conditioning episodes of ischaemia and reperfusion can be applied prior to the index ischaemic episode (ischaemic preconditioning), after the onset of the index ischaemic episode (ischaemic perconditioning), or at the onset of reperfusion (ischaemic postconditioning). Elucidation of the signal transduction pathways underlying ischaemic conditioning has identified a variety of pharmacological agents that are capable of reproducing its cardioprotective actions. Despite a wealth of preclinical, experimental animal data demonstrating clear cardioprotective benefits with these treatment strategies, their translation into clinical therapy has been hugely disappointing. This review explores the potential reasons behind this failure; it will focus primarily on the inadequacy of the experimental animal disease models that are currently being used to investigate novel cardioprotective strategies, which on the whole are not adequately representative of the clinical scenario, and finally, we will discuss potential solutions to remedy this problem.

## Introduction

Since the realisation that cardiomyocyte death was not inevitable following coronary artery occlusion, the search has been on for techniques or pharmacological agents that are capable of limiting myocardial infarct size. A major conceptual leap forward was the discovery that restoring coronary artery blood flow following an acute occlusion could limit ischaemic myocardial injury (Maroko et al., 1972); this is a finding which is still, currently, the optimal therapeutic strategy for an acute coronary artery occlusion. However, the process of reperfusing ischaemic myocardium is a 'double-edged sword' (Braunwald and Kloner, 1985) and can, in itself, induce cardiomyocyte death; this concept is known as lethal reperfusion injury (reviewed by Yellon and Hausenloy, 2007). The advent of interventional strategies and pharmacological agents that are capable of limiting myocardial injury when applied either before/during myocardial ischaemia or at the onset of myocardial reperfusion has demonstrated the potential application for cardioprotection. Despite the ability to demonstrate this enhanced cardioprotection in a variety of experimental animal disease models, the translation to the human clinical setting has been largely disappointing (Bolli et al., 2004; Kloner and Rezkalla, 2004). In this Commentary, we examine the shortcomings of the animal experimental disease models that are currently being used in this area of research and highlight areas where more representative animal disease models exist. Finally, we examine the design of clinical cardioprotection studies in order to increase the overall success in translating cardioprotective treatment strategies from 'bench to bedside'.

## Current animal models used to investigate myocardial ischaemia-reperfusion injury

Applying one or more brief non-lethal episodes of ischaemia and reperfusion to the heart itself (ischaemic conditioning), or to an organ or tissue away from the heart (remote ischaemic conditioning), has been widely reported to dramatically reduce the size of a myocardial infarct by 40-50% in a variety of different animal disease models. The 'conditioning' non-lethal episodes of ischaemia and reperfusion are able to elicit cardioprotection when applied prior to the index myocardial ischaemia (ischaemic preconditioning) (Murry, 1986; Przyklenk et al., 1993), after the onset of index myocardial ischaemia (ischaemic perconditioning) (Schmidt et al., 2007), or even at the onset of myocardial reperfusion (ischaemic postconditioning) (Kerendi et al., 2005; Zhao et al., 2003a; Zhao et al., 2003b; for further details of this interventional strategy, see the accompanying article by Mewton et al. in this issue of *DMM*).

Isolated, in vitro, buffer-perfused animal hearts have been the mainstay for investigating potential treatment strategies for protecting the heart against acute ischaemia-reperfusion injury.

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These hearts, which are subjected to regional or global ischaemia and reperfusion, can be used to determine cardiac enzyme release, infarct size or cardiac function. This animal disease model provides a robust, reproducible and efficient infarct model in which treatment strategies can be administered prior to infarction, during myocardial ischaemia or at the time of myocardial reperfusion. In vivo animal models of acute myocardial infarction (AMI), which may be technically more demanding, take into account the effects of an intact nervous system and circulation. However, on a number of different levels, these animal disease models cannot be expected to be representative of the complex human clinical setting of an AMI.

The response of the heart to acute myocardial ischaemia-reperfusion injury varies depending on the animal model of myocardial infarction used. For example, in rodent models of myocardial infarction, 30–40 minutes of either regional or global myocardial ischaemia is sufficient to induce an infarct size of 50% of the area at risk. However, in the porcine heart, longer durations (60–90 minutes) of myocardial ischaemia are required to achieve equivalent levels of infarction. Human myocardial infarcts generally require 90 minutes to become established, with maximum salvage possible before 6 hours and little benefit derived from reperfusion beyond 12 hours. Primate models can be used (principally macaque monkeys) which, although more representative of the human physiology, are prohibitively expensive. In these experimental models, short periods of myocardial reperfusion are used ranging from 1 to 2 hours for the in vitro studies, and from 2 to 72 hours for the in vivo infarct studies. The long-term effect of the cardioprotective treatment strategy, in terms of long-term infarct size reduction, cardiac remodelling and mortality, remains undetermined. However, the availability of echocardiography and small animal cardiac magnetic resonance imaging (MRI) has been used recently to delineate the effects of cardioprotective strategies on these cardiac indices, thereby making the studies more clinically relevant.

Experimental coronary artery occlusion in animal models of infarction is often mediated by external compression of a healthy coronary artery, whereas in the AMI patient, the rupture of an unstable atherosclerotic plaque and the formation of thrombus is responsible for the acute coronary occlusion. In addition, an AMI generates an acute inflammatory state that is not easily emulated in experimental animal models of infarction. Some animal disease models have attempted to mimic the scenario of an AMI in both rodents and larger animals. For example, endothelial irritants, such as ferric chloride, can be applied within a coronary artery (Dogne et al., 2005; Kurz et al., 1990) in order to activate the clotting cascade. In addition, thrombotic promotion can be achieved by using agents such as rose Bengal, or by green light laser agitation (Ikeda and Umemura, 2001), a hypercholesterolaemic diet or, in the carotid artery, by partial surgical ligation (Ishii et al., 2006). All of these techniques go some way to replicate the human scenario but they are challenging to perform, are not used widely, and are unable to fully mirror the complex interplay of events occurring in AMI. Closed chest, catheter and balloon coronary artery occlusion have all been used successfully (Olea et al., 2006; Toma et al., 2008) but require greater operator ability and are easiest in large animals. Given the facilities that are needed, this is not possible in all laboratories and using smaller animals is even more technically challenging.

## Impact of co-morbid illnesses on cardioprotection

The phenotypic patient suffering an AMI is male, aged 65 years old, and has a combination of co-morbid illnesses, which can include hypertension, diabetes, metabolic syndrome, hyperlipidaemia, and so forth. However, the majority of animal disease models are performed on young, healthy male animals in the absence of any co-morbid illnesses. It is well established that age, gender and the presence of disease states, such as hypertension, diabetes, metabolic syndrome, atherosclerosis and hyperlipidaemia, can impact on how the heart responds to cardioprotective treatment strategies (reviewed by Ferdinandy et al., 2007). In general, the presence of such a co-morbid illness renders the myocardium resistant to cardioprotection against infarction by physical or pharmacological stimuli; however, in some cases, cardioprotection may still be possible but a stronger cardioprotective stimulus is required to achieve an effect (Tsang et al., 2005a). Therefore, the development of novel cardioprotective treatment strategies should involve rigorous preclinical testing in animal disease models that take these factors into account. However, these animals are often significantly more expensive, and to investigate an agent in each disease setting would be difficult, although it would be easier to achieve using a collaborative strategy between different research groups. However, deficits in these individual models are evident as the combination of risk factors that is normally present in a CHD patient is difficult to replicate in one animal disease model.

## Translational experimental models

Testing a cardioprotective agent in human heart tissue, or a controlled human in vivo study, is a crucial translational step between bench and bedside. In this respect, using isolated human cells is an obvious advance from animal cardiac cells, although obtaining human cardiomyocytes is not easy. A further advance is the human atrial trabeculae model pioneered by our laboratory (Walker et al., 1995), in which human atrial trabeculae are isolated from right atrial appendage tissue, harvested from patients undergoing cardiac surgery, and are subjected to simulated ischaemia-reperfusion injury. This in vitro model provides a method for investigating a variety of cardioprotective strategies in human cardiac tissue that has been exposed to all the risk factors contributing to CHD; although, of course, ventricular muscle would be preferable and the end point of developed contractile force is a surrogate for myocardial injury. A variety of surrogate models exist for investigating the signalling mechanisms underlying ischaemic conditioning in human volunteers and patients, including models of endothelial function, exercise testing, coronary angioplasty, and so on (reviewed by Yellon and Hausenloy, 2005).

## Designing appropriate clinical cardioprotection trials

In the enthusiasm to demonstrate cardioprotection in the clinical setting, many interventions are rushed in to the clinical environment with conflicting or, at worst, no consistent evidence of benefit in animal studies. A working group convened by the US National Heart, Lung and Blood Institute (NHLBI) concluded that, before large expensive studies are undertaken in humans, animal studies should be conducted that are more akin to their clinical counterparts, with convincing benefit shown in multicentre, randomised, blinded controlled studies (Bolli et al., 2004).

Furthermore, choosing the appropriate clinical setting and treatment strategy is crucial, and it is imperative to take heed of the preclinical animal data in this respect. For example, overwhelming preclinical animal data suggested that cariporide (a sodium-hydrogen ion exchange inhibitor) was beneficial if administered before the index myocardial ischaemia, as opposed to prior to myocardial reperfusion (reviewed by Avkiran and Marber, 2002). Therefore, it was no surprise when clinical studies demonstrated that this agent was cardioprotective in the setting of cardiac surgery because, in this setting, the index ischaemic event can be anticipated, enabling cariporide to be given before ischaemia. However, cariporide was not effective in ST-elevation myocardial infarction (STEMI) patients undergoing primary percutaneous coronary intervention (PCI) because, in these patients, the index ischaemic event had already begun and cariporide has to be given before reperfusion (Theroux et al., 2000). Similarly, if the preclinical data suggest that a treatment strategy is effective only if administered either prior to, or at the onset of, myocardial reperfusion, there is little rationale for designing a study in which the drug is administered several hours following myocardial reperfusion.

Patient selection is crucial when designing clinical trials of cardioprotection strategies as adjuncts to myocardial reperfusion in STEMI patients. In this clinical setting, the ability to reduce infarct size and restore cardiac function can be used as an end point to assess the efficacy of cardioprotective strategies. This is similar to the methods that are used to evaluate animal myocardial infarction models. In STEMI patients, the acute rupture of an unstable coronary atherosclerotic plaque precipitates a complete acute thrombotic occlusion within the coronary artery, resulting in acute myocardial ischaemia. In this situation, the most effective intervention for salvaging viable myocardium is to reperfuse the coronary artery, using either thrombolysis to dissolve the thrombus or primary PCI to remove the occlusion. It has been suggested that 75% of STEMI patients obtain the maximum benefit with prompt myocardial reperfusion alone, leaving only 25% who may actually benefit from an adjunct to myocardial reperfusion (Miura and Miki, 2008). The current challenge is to identify, and target, this high-risk group of patients with adjunctive reperfusion therapy in order to enhance myocardial salvage. In terms of myocardial salvage, STEMI patients presenting for myocardial reperfusion within 0 to 6 hours of chest pain accrue the most benefit. Whether the same applies to a cardioprotective strategy designed to attenuate lethal reperfusion injury is currently unclear. However, the current clinical evidence suggests that the patients who are most likely to benefit from a cardioprotective treatment strategy, administered at the onset of myocardial reperfusion, are those sustaining a large myocardial infarct (usually an anterior infarct) in which the infarct size is greater than 40% of the left ventricular volume. Presumably, in this group of patients, the contribution of lethal reperfusion injury is significant and so administering an adjunct to myocardial reperfusion is beneficial.

If the cardioprotective strategy needs to be administered before, or at the immediate onset of, myocardial reperfusion, there is little rationale for recruiting patients who do not have a fully occluded coronary artery [thrombolysis in myocardial infarction (TIMI) <1] at presentation. However, it is appreciated that the

infarct-related coronary artery may spontaneously occlude or open before primary PCI, in which case, this can be monitored by continuous in-ambulance electrocardiographic (ECG) monitoring. Other determinants of myocardial infarct size that need to be excluded include the area at risk (which can be estimated using coronary angiography, nuclear myocardial scanning or, more recently, cardiac MRI), the presence of significant collateralisation to the area at risk (which can be excluded by Rentrop grading at the time of coronary angiography) and the presence of chest pain within 3 days (which may inadvertently precondition the patient before their myocardial infarction).

The end points of cardioprotection have to be carefully chosen in these clinical cardioprotection studies and, for proof-of-concept studies, these have often been restricted to the measurement of cardiac enzymes. However, given the large numbers of patients that are required for the definitive mortality studies, more robust end points of clinical cardioprotection are required as surrogate markers of clinical outcome. In this respect, cardiac MRI is emerging as a powerful imaging strategy that is capable of quantifying left ventricular dimensions and function, myocardial infarct size, the area at risk, microvascular obstruction and myocardial haemorrhage, which are all indices that have been linked to clinical outcomes following an AMI (Lockie et al., 2009). The improved spatial resolution of MRI over and above nuclear imaging enables greater reproducibility and allows smaller sample sizes to be used in clinical trials. In addition, a number of different measures of cardioprotection can be made within one imaging modality, increasingly making MRI the modality of choice in assessing surrogate outcomes of cardioprotection.

### Cardioprotection not lost in translation

There have been a couple of recent examples in which the transition from bench to bedside has been successful. Interrupting myocardial reperfusion with several short-lived episodes (between 5 and 60 seconds) of myocardial ischaemia and reperfusion has been demonstrated, in a variety of animal infarct models, to reduce myocardial infarct size by 40-50%; this phenomenon is known as ischaemic postconditioning (Hausenloy 2009; Tsang et al., 2005b; Zhao et al., 2003a). In the clinical setting, this has been achieved by performing three to six cycles of inflation and deflation of a coronary angioplasty balloon following deployment of the stent within the infarct-related coronary artery. This protocol has been reported to reduce myocardial infarct size at 6 months and preserve the left ventricular ejection fraction at 1 year (Thibault et al., 2008). Similarly, preclinical animal infarct data have established that pharmacologically inhibiting the mitochondrial permeability transition pore (mPTP), a non-selective channel of the inner mitochondrial membrane that mediates cell death by opening at the onset of myocardial reperfusion, reduces myocardial infarct size by 30-40% (Hausenloy et al., 2002; Hausenloy et al., 2003), preserves the left ventricular ejection fraction, and improves survival (Gomez et al., 2007). A recent proof-of-concept clinical study has demonstrated that, as an adjunct to primary PCI, cyclosporine A is capable of reducing myocardial infarct size in STEMI patients (Piot et al., 2008). Clearly, for both these treatment interventions, large multicentre studies are required to determine their effect on clinical outcomes.

## Conclusion

In order to advance the research field of cardioprotection and harness the huge potential of ischaemic conditioning, cardiovascular scientists and cardiologists will have to work side by side to ensure that (1) the design of suitable, preclinical animal disease models more accurately reflects the clinical setting under scrutiny and (2) the design of clinical cardioprotection trials takes into account the major findings of the laboratory studies. Through this integrated and co-ordinated approach, the carefully selected cardioprotective interventions should be investigated in the clinical arena using rigorously designed proof-of-concept clinical studies followed by larger studies using surrogate markers of clinical end points (such as cardiac MRI), before multicentre clinical evaluation of clinical outcomes.

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## COMPETING INTERESTS

The authors declare no competing financial interests.

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