Targeting the Reperfusion Injury Salvage Kinase Pathway in the Clinical Setting

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Declaration:

I declare that the work presented within this thesis is entirely my own. Where elements of the work have been aided this is clearly stated. The thesis presented is the one on which I expect to be examined.

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Abstract

Cardiovascular disease remains the leading cause of morbidity and mortality worldwide. Ischaemic heart disease contributes the largest burden and despite advances in revascularisation therapy significant morbidity and mortality exist in both the elective and emergency treatment setting.

Short episodes of sub-lethal ischaemia and reperfusion applied before a period of prolonged ischaemia (preconditioning) and reperfusion stuttered with short episodes of ischaemia (postconditioning) are powerful, endogenous, cardioprotective phenomena which offer the potential to reduce myocardial injury and infarction by as much as 50%. Despite many years of research these mechanical, invasive techniques have not been adopted to routine practice. A pharmacological mimetic, targeting the same protective pathways as pre- and post-conditioning would have great potential in reducing myocardial injury in a number of clinical settings and could be easily administered and adopted to the clinical arena.

Chapter 1 of this thesis summarises the research to date in this rapidly evolving field concentrating on two pharmacological conditioning mimetics, atorvastatin and erythropoietin and the clinical assessment of cardioprotection and myocardial salvage. Chapter 2 details the hypotheses to be investigated. Chapter 3 describes two studies undertaken in coronary artery bypass surgery with high dose atorvastatin as a potential cardioprotective agent. Chapter 4 describes a study testing the use of erythropoietin in patients presenting with acute myocardial infarction requiring emergency angioplasty and using cardiac magnetic resonance outcome measures. Chapter 5 highlights the difficulties in translating pre-clinical animal studies to the human clinical setting and discusses potential methods to improve this.

In summary, this thesis examines the pre-existing research regarding atorvastatin and erythropoietin as cardioprotective agents. Novel clinical studies testing the use of these agents in the settings of coronary artery bypass surgery and acute myocardial infarction are presented. The findings are discussed and reviewed in the context of ongoing advances in the field of cardioprotection.

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Acknowledgements

CHAPTER 1

Introduction

Ischaemic Heart Disease

Ischaemic heart disease (IHD) is the leading cause of mortality worldwide and some predict that this will remain the case at least as far ahead as 2020[1]. Similarly in the United Kingdom (UK) cardiovascular disease is the leading cause of death accounting for almost 198,000 deaths each year, with 94,000 directly attributable to IHD[2]. Importantly IHD is one of the leading causes of premature mortality causing almost 31,000 premature deaths in the UK in 2006. IHD is estimated to cost the UK economy approximately £9 billion per annum in direct health care costs, loss of productivity and informal care[3].

It is the formation of atheroma, lipid rich deposits, within the epidcardial coronary arteries that causes IHD. The reduction in arterial diameter may lead to a slow but progressive increase in symptoms or an atheromatous plaque may rupture suddenly leading to thrombotic occlusion of the coronary artery and the most notable manifestation of IHD, acute myocardial infarction (AMI)[4]. There are approximately 146,000 AMIs in the UK each year[5] and without urgent treatment AMI may either lead to death from cardiac arrhythmia/failure/rupture or more often will result in substantial morbidity through the development of chronic cardiac failure. Current treatments for cardiovascular disease are directed at primary prevention of ischaemic events, elective procedures (percutaneous coronary intervention [PCI] or coronary artery bypass graft [CABG] surgery) to ensure adequate myocardial blood supply, emergency treatments to re-establish blood flow in occluded coronary arteries (thrombolytic agents,

primary PCI or emergency CABG surgery) and following this secondary prevention of further ischaemic episodes[6].

Despite advances in current treatments the ongoing mortality figures above suggest that improvements are still urgently needed in all areas. In this regard the field of 'cardioprotection' and in particular 'myocardial conditioning' may be able to offer benefits in a wide range of clinical scenarios.

Myocardial Ischaemia and Reperfusion Injury

Cessation of blood supplying oxygen and nutrients to the myocardium (ischaemia) results in a cascade of metabolic changes with resultant implications for cellular processes. If the blood supply is not re-established within a matter of minutes irreversible cell death may occur. However, following prolonged ischaemia the process of re-establishing the blood supply (reperfusion) with attempted rapid resolution of the normal biochemical and metabolic state, may itself initiate cell signalling pathways responsible for further myocyte death[7]. The relative contributions to myocardial injury of ischaemia or reperfusion are difficult to quantify. However, in the majority of clinical settings where myocardial injury is evident, that injury arises from a combination of both ischaemia and reperfusion[8].

Ischaemic injury

During ischaemia, hypoxia develops resulting in anaerobic metabolism which in turn contributes to a rising intracellular acidosis. In response to the acidosis, hydrogen ions are pumped out of the cell by the H^+/Na^+ exchanger leading to an excess intracellular Na^+. The raised Na^+ is dealt with by the Na^+/Ca^{2+} exchanger which leads to a progressive rise in intracellular Ca^{2+}.[9] Cellular ATP is progressively depleted, ion pumps cannot function resulting in further intracellular calcium rise and ATP is further reduced. There may be

additional Ca^{2+} release from the sarcoplasmic reticulum. Progressive drop in cell pH and cytosolic swelling threaten to cause plasma membrane rupture. Plasma membrane rupture is further threatened by caspase mediated cleavage of structural proteins in the cytosolic membrane. Plasma membrane rupture commits the cells to necrotic death and the accompanying destructive inflammatory response, however caspase mediated cleavage of essential proteins can also trigger apoptotic cell death[8].

Lethal myocardial reperfusion injury

At reperfusion, oxygen is reintroduced and an attempt is made to re-establish normal physiologic parameters. However the abrupt biochemical and metabolic changes which occur are detrimental to the myocardium and compound the damage which has commenced during the ischaemic period.

Reactive Oxygen Species (ROS) are produced from the re-energised mitochondria, from endothelial xanthine oxidase and later in the reperfusion period from neutrophilic NADPH oxidase. ROS contribute to myocardial injury by acting as neutrophil chemoattractants, causing sarcoplasmic reticulum dysfunction, exacerbating intracellular calcium overload, damaging the cell membrane by lipid peroxidation, inducing enzyme denaturation, via oxidative damage to DNA and by inducing the opening of a conformational transmembrane mitochondrial pore, termed the mitochondrial permeability transition pore (mPTP). The already calcium overloaded cardiomyocyte is subject to further calcium loading at reperfusion due to a damaged sarcolemmal membrane, dysfunctional sarcoplasmic reticulum and reverse function of the sodium-calcium exchanger. Resumed ATP generation in the presence of calcium overload causes hypercontracture which may induce cell death. The rapid normalisation of pH facilitates mPTP opening and contributes to cardiomyocyte hypercontracture. The restoration of the mitochondrial membrane potential forces calcium in

to the mitochondria which in conjunction with ROS and the restoration of normal pH act to open the mPTP. This opening uncouples oxidative phosphorylation and induces mitochondrial swelling which if mitochondrial integrity is disrupted may cause the release of substances such as cytochrome C and cell death is likely to be inevitable[10]. Later in the reperfusion process neutrophils accumulate in the myocardium in response to chemoattractants - they may promote cell death by further ROS generation, the release of degradative enzymes and vascular plugging[7 11].

Mechanisms of myocyte cell death

It appears that necrosis plays the major role in myocyte cell death during ischaemia-reperfusion injury (IRI) but that apoptosis or 'programmed cell death' also makes a contribution. The more newly described cellular function of autophagy may also play a part but its precise contribution is not clear. Necrosis is characterised by cell swelling with membrane rupture leading to a release of cytosolic components and an inflammatory reaction. Although classically thought of as an unregulated process, it is now becoming apparent that in fact necrosis does have a predictable course and is probably triggered and regulated by the cell in response to local changes[12]. Apoptosis, as originally described by Kerr et al.[13], results in chromatin condensation and fragmentation, cell shrinkage and plasma membrane budding with the release of apoptotic bodies which contain cellular components. These in turn are phagocytosed with little or no inflammatory response. Autophagy degrades and recycles cytoplasmic components and selectively removes damaged mitochondria as a cytoprotective mechanism. It does play a role in IRI although it is not yet established whether cells may die 'with autophagy' or 'by autophagy'[14] or in fact whether autophagy as an adaptive response may in some part play a protective role from IRI.[15]

Cell death during ischaemia, at, and following reperfusion is probably a combination of necrosis, apoptosis and autophagy. The three mechanisms have been reported to be inter-related and as such one can use the encompassing term 'cell death' to more easily describe these processes. Importantly all three mechanisms are now thought to be heavily regulated and as such can be targeted in order to attempt to increase cell survival. By inhibiting or promoting key elements in the pathways leading to 'cell death', interventions probably impact on all three mechanisms but with the same overall aim of reducing cell death and promoting cell survival.[12]

Myocardial conditioning

Cardioprotection has been defined as, "all mechanisms and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage"[16]. The term 'myocardial conditioning' is encompassed within cardioprotection and is a broad description of agents or techniques which act to 'condition' and subsequently protect the myocardium by activating endogenous cardioprotective mechanisms. The concept of being able to reduce damage to the myocardium evolved from a number of investigators in the 1960's, but initial disparate findings were summarised by Braunwald in 1974[17], who suggested that the time for prospective trials in this new area had arrived.

Ischaemic preconditioning

Ischaemic preconditioning (IPC) is a powerful endogenous cardioprotective stimulus which has the ability to reduce myocardial infarct size. First described in a canine model of infarction by Murry et al.[18] in 1986, IPC is a physical cardioprotective technique in which short sub-lethal bursts of myocardial ischaemia and reperfusion provide protection against a subsequent, potentially lethal, episode of myocardial ischaemia and reperfusion. This seminal

study paved the way for an explosion of research investigating the mechanism of protection and trying to replicate the protective effect at different time points using pharmacological agents (termed pharmacological preconditioning). IPC was shown to be protective in a variety of species such as pigs[19], rabbits[20], rats[21] and mice[22] and was subsequently reported to be protective in humans as well[23]. The protection afforded by IPC appears to be biphasic in nature with an early phase (classical or acute IPC) providing protection for approximately 2-3 hours and a delayed phase (Second Window of Protection-SWOP) providing protection (albeit less potent) after approximately 12-24 hours and for up to 72 hours[24] [25] . The application of IPC in humans has been limited however due to its interventional nature and by the fact that the protective stimulus should be applied prior to the potential myocardial injury, which in the case of acute myocardial infarction is difficult to predict. In this case an intervention that could be applied after the onset of ischaemia would be more practicable.

Ischaemic postconditioning

Ischaemic postconditioning (IPost) is a technique whereby the restoration of reperfusion is stuttered by repeat transient episodes of ischaemia following a period of lethal ischaemia. Interestingly in 1994, a human case report describing the cessation of reperfusion arrhythmia in acute MI by re-occlusion of the coronary artery was published[26] although the mechanisms behind this phenonmenon were the subject of discussion only. The first study to coin the term 'postconditioning' was reported by Na et al. in 1996[27]. Using a feline model of ischaemia and ventricular premature beat driven interrupted reperfusion they demonstrated a significant reduction in the incidence of reperfusion associated ventricular fibrillation. Subsequently hypoxic post-conditioning was reported to reduce injury to rat cardiomyocytes subjected to hypoxia and reoxygenation[28], however the now more widely accepted method of ischaemic postconditioning (IPost) was described in 2003 in a canine model by Zhao et al.[29] The

investigators occluded the LAD for 60 minutes and then prior to 3 hours of continued reperfusion, the artery was unclamped and reclamped for 3 cycles of 30 seconds, therefore stuttering the reperfusion phase. It was demonstrated that postconditioning was able to reduce infarct size to the same extent as ischaemic preconditioning. Applied at the onset of or just after reperfusion, this technique activates very similar cell survival pathways to IPC (see later) and produced the exciting possibility of being able to intervene in humans after the onset of ischaemia and realise large myocardium saving potential. This has been tested in the human setting of acute MI by a number of investigators. In the first study, Ovize's group took 30 patients with ongoing acute myocardial infarction who were due to be reperfused by primary coronary angioplasty. Following direct stenting, in the intervention group, reperfusion was immediately interrupted by 4 cycles of 1 minute balloon inflation and deflation to impede flow in the coronary artery; the control group underwent reperfusion only[30]. Using total creatine kinase (CK) over 72 hours following the myocardial infarction as a surrogate marker of myocardial infarct size, the authors reported a 36% reduction in infarct size in the intervention group. In another study Laskey used two 90 second balloon inflations as the conditioning protocol in 10 patients undergoing emergency angioplasty for acute MI, the control group (7 patients) had only one balloon inflation of 90 seconds. Despite the unusual protocol, it was demonstrated that the postconditioned group experienced improved ST segment resolution and coronary blood flow[31]. Ma et al. used 3 cycles of 30 seconds ischaemia and reperfusion as the post-conditioning protocol in a study of 94 patients with acute MI. They demonstrated that IPost improved coronary arterial flow, improved endothelial function and reduced cardiac enzyme release[32]. Following these initial proof of concept studies, Ovize's group went on to recruit a new cohort of 38 patients and using the same postconditioning protocol demonstrated that postconditioning significantly reduced myocardial enzyme release using nuclear imaging they showed a reduction in mean infarct

size from 19.5% to 11.8%. At 1 year of follow-up echocardiography demonstrated a 7% improvement in left ventricular ejection fraction in the intervention group, proving the longevity of the postconditioning benefits[33]. Recently 118 patients randomised to usual care or who underwent post-conditioning (4 cycles of 30 seconds of balloon inflation/deflation) following reperfusion with PPCI for STEMI were evaluated with cardiac MRI and the postconditioning group had a 19% relative reduction of infarct size (51+/-16% of total area at risk versus 63+/-17%, P<0.01), corresponding to a 31% increase in salvage ratio[34].

Disappointingly these impressive findings have not yet been validated in a large multi-centre study and there has been a reluctance to adopt the technique due to its interventional nature and concerns at prolonging the patients' procedure. Thus the ability to give a pharmacological agent to a patient, prior to, during or following ischaemia, capable of replicating the protective effects of pre- or post-conditioning would be a huge leap forward not only in demonstrating a cardioprotective effect, but a safe pharmacological agent is much more likely to be adopted in to the clinical arena and would have widespread potential for use wherever myocardial injury is anticipated or ongoing.

Pharmacological Conditioning

Since the realisation that it was possible to intervene and reduce myocardial infarction size, many different pharmacological agents have been experimented with in order to try and increase myocyte survival. Careful experimental elucidation of the cell signalling pathways involved in pre and post conditioning has identified targets for pharmacological agents to act as mimetics of the conditioning stimulus. Unfortunately, despite more than 20 years of research, no pharmacological agent has shown consistent benefits and been adopted to routine clinical practice. There are many reasons for this 'failure of translation' and these shall be described in more detail later (chapter 5), however it is only through continued

rigorous laboratory research using clinically-relevant experimental models to elucidate the underlying cardioprotective mechanisms, followed by carefully designed and targeted clinical studies, that a successful cardioprotective agent will be found.

Improving our understanding of the cardioprotective cell signalling pathways implicated will assist in improving the chance of successful translation from laboratory to the clinical setting. The actual pathways underlying IPC and IPost remain unclear although the current paradigm is summarised below.

Potential mechanistic pathway underlying ischaemic preconditioning

As described above the physical cardioprotective stimulus of IPC reduces eventual infarct size in the face of ischaemia – reperfusion.

IPC acts to increase circulating serum levels of endogenous mediators which are either able to act via cell surface receptors or act independently. Acting via G protein coupled receptors on the cell surface the main triggers of IPC are adenosine[35], bradykinin[36] and opioids[37]. Receptor-independent triggers are thought to include nitric oxide as its blockade decreases IPC in rats[38], free radicals as although harmful to a certain degree are able to activate G-proteins, protein kinases and K_{ATP} channels[39] and the calcium L-type channel which when blocked prevents IPC[40]. The signalling cascade appears to converge on PKC as a pivotal point[41] although the role of differing isoforms is still being investigated[42]. The upstream signalling from PKC is thought at present to be via phosphatidylinositol-3-OH kinase (PI3K)-AKT, endothelial nitric oxide synthase (eNOS), cGMP and PKG which culminates in phosphorylation and opening of the mitochondrial K_{ATP} channel[43]. Via the mitochondrial electron transport chain a burst of reactive oxygen species are produced which serves to further activate a number of pro-survival kinases including p38, JNK, extracellular signal-

related kinase (ERK), PKC, AKT and other mitogen activated protein kinase (MAPK)[44] resulting in cardioprotection. Many of these survival kinases activate nuclear transcription factors and mediators of delayed preconditioning.

The Reperfusion Injury Salvage Kinase (RISK) pathway

Given that the anti-apoptotic survival kinases PI3K-AKT and ERK were shown to have such a pivotal role in IPC and that apoptosis contributes to myocardial infarct size, Yellon and Baxter[45] first formulated and tested the hypothesis that pharmacological activation of these kinases may protect the myocardium against reperfusion injury. In fact, it was subsequently shown that both necrosis and apoptosis were limited by activation of this pathway resulting in a reduction in infarct size[46]. Following this it was demonstrated that IPC resulted in phosphorylation and activation of the PI3K-AKT and ERK signalling pathways not only at the time of the initial IPC stimulus but at the time of reperfusion as well[47]. Blockade of PI3K-AKT and ERK signalling pathways abrogated the protection afforded by IPC and confirmed them as crucial to the infarct size reduction[46]. These pathways were subsequently termed the Reperfusion Injury Salvage Kinase (RISK) pathway[48].

The method in which IPC actually activates the RISK pathway at reperfusion is still under investigation. It is postulated that the initial IPC stimulus may either prime the kinases or enable their cellular redistribution. Alternatively there may be an intermediary which acts at reperfusion after the delay. The intermediary may well be PKC which is able to act up to 3 hours following the IPC stimulus[49].

End effectors of the RISK pathway and the mPTP

The RISK pathway is thought to act downstream by inhibiting pro-apoptotic factors, inhibiting GSK3-β, activating eNOS, and via the mitochondrial translocation of PKCε[50]. Pro-

survival pathways appear to converge on a common target for cell survival, the mitochondrial permeability transition pore (mPTP). Inhibition of the mPTP in the time following reperfusion enables the mitochondria to retain stability, promoting cell survival and limiting reperfusion injury[51].

Importantly for translation to the acute clinical setting it is also possible to harness the protective effects of the RISK pathway with pharmacological or physical interventions applied at the time of reperfusion (IPost) without the need to have applied a pre-ischaemic stimulus.

Cell signalling pathways activated by postconditioning

As described above the technique of postconditioning[29], which is a modified form of reperfusion, is also able to reduce the eventual infarct size resulting from ischaemia-reperfusion injury.

It appears that postconditioning is able to recruit the RISK pathway at reperfusion to exert its cardioprotective effect[52]. The postconditioning stimulus is able to activate both PI3K-AKT and ERK1/2 via a diverse range of receptors including GPCRs and those for growth factors[46]. Levels of nitric oxide (NO) are increased via eNOS and act on PKG, and probably via PKC in order to activate the mitochondrial K_{ATP} channel which in turn inhibits the opening of the mPTP. Increased levels of NO as well as glycogen synthase 3β (GSK 3β) are thought to be able to inhibit the mPTP directly[53]. In this way both the cardioprotective phenomena of IPC and IPost are postulated to converge on the mPTP as a crucial unifying mediator of the cell survival signal[54].

Potential targets for pharmacological cardioprotective agents

Elucidation of the survival mechanisms described above has allowed the identification of a number of pharmacological agents that, by activating parts of the described pathway, are hypothesised to be able to limit infarct size (figure 1). Two such agents are the HMG-CoA reductase inhibitors (henceforth referred to as 'statins') and erythropoietin; which are thought to act via the RISK pathway to a common end point of inhibiting the mPTP and reducing myocardial cell death. The next sections shall review the use of these two agents to date.

Figure 1: Acting at reperfusion via the RISK pathway, erythropoietin and atorvastatin are two examples of pharmacological mimetics of ischaemic pre/post conditioning. Below is a simplified schematic of the potential protective effect of atorvastatin and erythropoietin. Thought to act via GPCR on the cell membrane, at reperfusion the RISK pathway is triggered. Increased intracellular NO along with a small burst of ROS is proposed to inhibit the k_{ATP} ion pump and subsequently inhibit mPTP opening. By inhibiting mPTP opening mitochondrial stability is maintained, cardiomyocyte survival promoted and resultant myocardial infarction size is reduced. [RISK: Reperfusion Injury Salvage Kinase pathway, GPCR: G-protein coupled receptor, mPTP: mitochondrial permeability transition pore, NO: nitric oxide, ROS: reactive oxygen species].
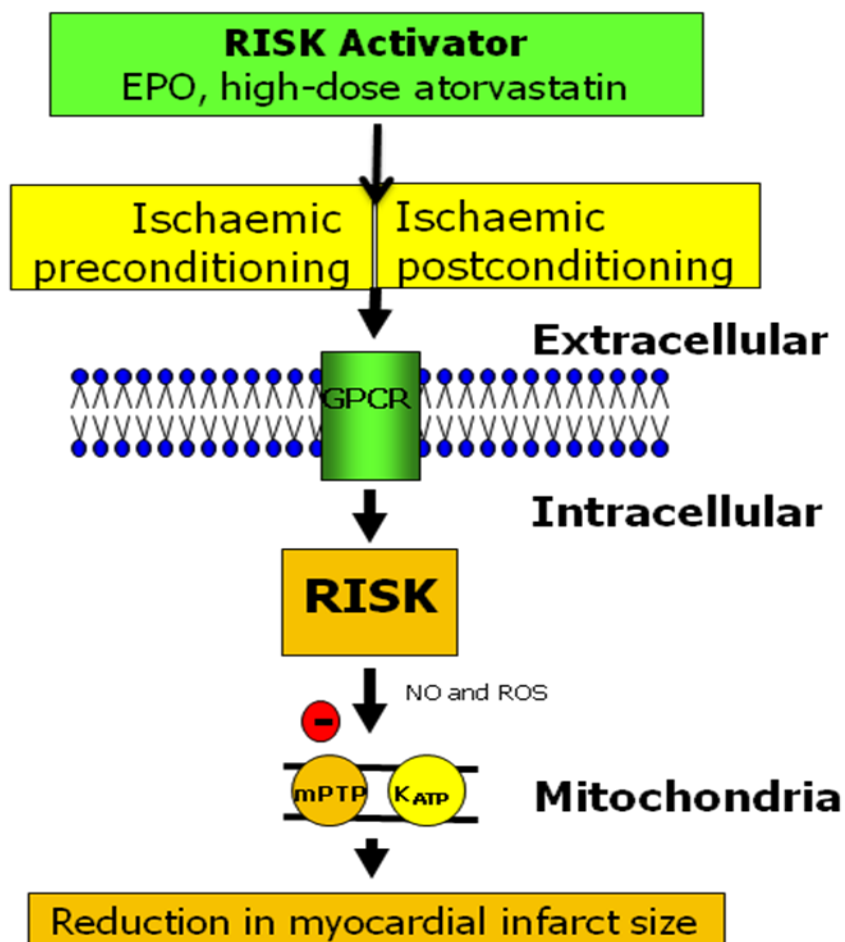
'Statins' as a myocardial conditioning mimetic

HMG-CoA reductase inhibitors have become standard medical therapy in the armamentarium available for the prevention and treatment of cardiovascular disease. Large randomised controlled clinical trials have established their role as effective medical therapy for the primary[55] [56] and secondary[57] [58] prevention of cardiovascular events. HMG-CoA reductase inhibitors competitively inhibit the conversion of acetyl coenzyme A and acetoacetyl coenzyme A to mevalonate in the formation of cholesterol and prevent the formation of the isoprenoids[59] (see figure 2). Statins were first developed in order to lower total serum cholesterol and improve the lipid profile but have subsequently been shown to exert a variety of beneficial, 'pleiotropic' effects, particularly relevant to cardiovascular disease, including improved endothelial function, reduced oxidative stress, less platelet adhesion, and atherosclerotic plaque stabilisation[60].

It is well-established that statin therapy offers widespread beneficial effects on the cardiovascular system through both its lipid lowering and non-lipid lowering effects described above. However, there is a less appreciated non-lipid lowering effect of statin therapy, namely its potential to directly protect the myocardium from the detrimental effects of acute ischaemia-reperfusion injury, a feature which has been widely documented in the experimental literature.

Figure 2: The HMG-CoA reductase inhibitors or Statins prevent the formation of mevalonate and the isoprenoids.
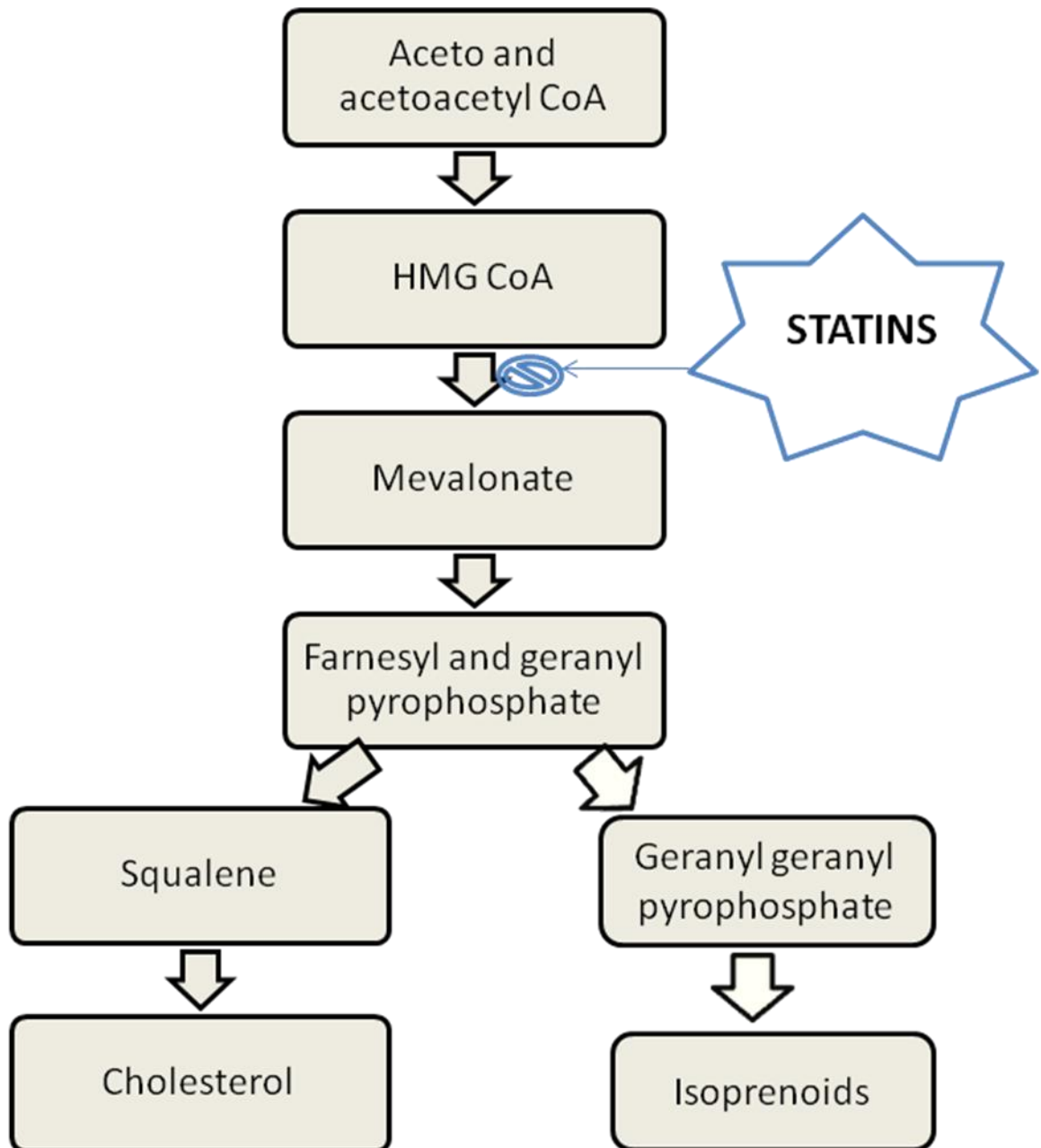
Experimental cardioprotection using statin therapy

In the late 1990s, the finding that statin therapy was beneficial in terms of improving cardiovascular outcomes resulted in a search for the mechanisms underlying this cardioprotective effect. Using experimental animal models of acute myocardial ischaemia-reperfusion injury, it is possible to examine the effects of statin therapy at different time-points in the course of the acute ischaemia-reperfusion insult. Clearly, the time-point at which the statin treatment is administered has a direct bearing on its potential for clinical application. For example, pre-ischaemic statin treatment would be limited to the clinical settings of planned cardiac surgery and elective PCI procedures in which the timing of the ischaemic can be readily anticipated.

Statin therapy administered prior to myocardial ischaemia

Initial studies in 1998 suggested that statin therapy may be able to exert direct cytoprotective effects through the upregulation of endothelial nitric oxide synthase (eNOS), a critical non-lipid lowering effect of statin therapy[61]. On this background, in 1999, the first experimental animal studies were published demonstrating direct cardioprotective effects elicited by statin treatment. Ueda and colleagues[62] investigated the effects of pravastatin pre-treatment on the response of the hypercholesterolaemic rabbit heart to the endogenous cardioprotective effects of ischemic preconditioning (IPC). Crucially this important study found that the presence of hypercholesterolaemia blunted the cardioprotective benefits of IPC. These authors were able to demonstrate that pravastatin could restore the infarct-limiting effects of IPC in hypercholesterolaemic hearts, and the mechanism underlying this effect was not associated with lipid-lowering and was attributed to the activation of ecto-5'-nucleotidase, an adenosine producing enzyme. Interestingly, in this study, pravastatin given alone without the added

stimulus of IPC did not reduce infarct size, suggesting perhaps that a sub-threshold dose of pravastatin had been used particularly as a later study has demonstrated cardioprotection with pravastatin alone[63]. Lefer and colleagues[64] demonstrated that pre-treatment with simvastatin was able to protect isolated perfused normocholesterolaemic rat hearts against acute ischemia-reperfusion injury. The authors reported that the cardioprotective effect was dependent on the presence of neutrophils and resulted from the reduction of the inflammatory response provoked by acute ischaemia-reperfusion injury (see table 1 for a summary of the experimental studies demonstrating pre-ischaemic cardioprotection with statin therapy).

Table 1: Experimental cardioprotection with Statin therapy given prior to myocardial ischaemia.

Study	Experimental Model	Treatment regime	Key results	Novel mechanistic insight
Pre-ischaemic cardioprotection elicited by Statin therapy				
Ueda et al 1999[62]	Hypercholesterolaemic perfused rabbit hearts	Pre-treatment for 8 weeks with Pravastatin 5 mg/kg/day (orally).	Restoration of IPC protection in hypercholesterolaemic hearts but not in normal ones, associated with ecto-5'-nucleotidase activity.	Synergistic cardioprotective effect with IPC.
Lefer et al 1999[64]	Perfused rat hearts.	Pre-treatment with 25µg Simvastatin or Pravastatin 18 hours prior IRI. Hearts perfused with PMNs.	↑LV developed pressure ↓PMN infiltration ↓CD18 upregulation in PMN. ↓PMN adherence to rat vascular endothelium. ↓P-selectin expression.	Anti-inflammatory effect dependent on NO.
Scalia et al 2001[65]	APO E-/- mice fed a high cholesterol diet subjected to in situ IRI.	Pre-treatment with a subcutaneous injection of 1 mg/kg Simvastatin 18 hours prior to IRI.	Reduced myocardial infarct size. No effect on cholesterol.	Simvastatin able to cardioprotect an atherosclerotic animal heart model. Anti-inflammatory effect dependent on NO

Study	Experimental Model	Treatment regime	Key results	Novel mechanistic insight
Lefer et al 2001[66]	In situ Db/Db murine heart	Pre-treatment for 5 days with intraperitoneal simvastatin (0.5mg/kg daily).	Reduced myocardial infarct size. ↓PMN infiltration ↓PMN adherence to rat vascular endothelium	Simvastatin able to cardioprotect a type II diabetic animal heart model. Anti-inflammatory effect dependent on NO.
Kawabata et al 2001[67]	Isolated rabbit hearts.	Pre-treatment with intravenous Pravastatin (0.025mg/kg) 60 minutes prior to IRI.	Preserved ATP levels and maintained pH (MRI spectroscopy). These effects abolished by glibenclamide and L-NAME.	Pravastatin-induced cardioprotection of rabbit heart via K_{ATP} channel and NO.
Di Napoli et al 2001[68]	Perfused rat hearts	Simvastatin acutely before and during IRI at 10, 25, 50 and 100 µM. 15mins I, 22-180mins R.	Optimal results seen with 25µM simvastatin. ↓CK in effluent. ↓vascular permeability. ↑eNOS mRNA and protein. ↓iNOS mRNA and protein.	Simvastatin provides cardioprotection via an eNOS dependent pathway.
Ikeda et al 2003[69]	Perfused rat hearts	Pre-treatment Rosuvastatin (0.25 or 1.25 mg/kg) given 18 hours prior to IRI.	↑LV developed pressure ↓PMN infiltration ↓PMN adherence to rat vascular endothelium	Rosuvastatin able to cardioprotect rat hearts. Anti-inflammatory effect dependent on NO.
Lazar et al 2003[70]	In situ pig heart	Pre-treatment with atorvastatin 40 mg daily given orally for 21 days	Less arrhythmias Improved wall-motion scores Smaller infarct size. No effect on cholesterol	Atorvastatin able to cardioprotect the pig heart when administered for 3 weeks.
Wolfrum et al 2003[71]	In situ rat heart	Pre-treatment with Cerivastatin (0.3 mg/kg/d) for one week.	Smaller infarct. Increased eNOS and cardioprotection blocked by L-NAME.	Cerivastatin able to cardioprotect the rat heart. Protection dependent on eNOS.
Tiefenbacher et al 2003[72]	In situ rat heart	Pre-treatment of Fluvastatin IV bolus (2mg/kg) given 20 minutes prior to IRI. followed by IV infusion of 1mg/kg/hr.	↑Regional wall thickening ↑myocardial blood flow and ↓Infarct size Protection abolished by L-NAME ↓myocardial MPO.	Fluvastatin able to acutely cardioprotect the rat heart. Anti-inflammatory effect dependent on NO.

Study	Experimental Model	Treatment regime	Key results	Novel mechanistic insight
Tavackoli et al 2004[73]	In situ rat heart	Pre-treatment Simvastatin (20 mg/kg per day) for 3 days.	Smaller infarct size, protection blocked by glyburide.	Simvastatin-induced cardioprotection of rat heart via K_{ATP} channel
Sanada et al 2004[74]	In situ dog heart	Pre-treatment of IV bolus of Pravastatin (0.2, 2, or 10 mg/kg), Pitavastatin (0.01, 0.1, or 0.5 mg/kg), or Cerivastatin (0.5, 5, or 50μg/kg) given immediately prior to IRI.	Smaller infarct size with protection blocked by wortmannin or 8-SPT given at reperfusion.	Cardioprotection of the canine heart requires activation of PI3K-Akt and ecto-5'-nucleotidase activity at reperfusion.
Verma et al 2004[75]	Cultured human ventricular cardiomyocytes subjected to simulated IRI.	Pravastatin (1, 10, and 100 μM) added to the hypoxic/reoxygenation buffers.	Reduced cardiomyocyte death. Protection blocked by bosentan, L-NAME and was associated with production of NO and Akt phosphorylation.	Pravastatin able to cardioprotect isolated human ventricular cardiomyocytes through ET-1, Akt and NO.
Birnbaum et al 2005[76]	In situ rat heart	Pre-treatment with atorvastatin 10 mg/kg/day (oral gavage) prior to IRI.	Smaller infarct size with protection blocked by COX-2 inhibitor. Protection associated eNOS and iNOS phosphorylation as well as prostaglandin production.	Cardioprotection of the rat heart requires COX-2 activation and prostaglandin release.
Di Napoli et al 2005[77]	Isolated rat hearts	Oral treatment with Rosuvastatin (0.2-20mg/kg) for 3 weeks.	Less myocardial dysfunction, endothelial dysfunction and mitochondrial damage.	NO dependent effects on vascular endothelium and myocardium.
Mensah et al 2005[78]	Isolated rat heart	Oral gavage for 1, 3 days and 1 or 2 weeks with oral 20mg/kg atorvastatin. Supplemental dose of 40mg/kg given prior to IRI.	Reduced infarct size with 1 or 3 days treatment but not 1 or 2 weeks treatment. Protection recaptured if acute atorvastatin 40mg given prior to IRI	Cardioprotective effect of atorvastatin wanes with chronic dosing possibly due to down-regulation of PI3K-Akt pathway by PTEN. Possible to recapture protection with acute high dose of atorvastatin.

Study	Experimental Model	Treatment regime	Key results	Novel mechanistic insight
Atar et al 2006[79]	In vivo rat	Oral gavage with 3 days atorvastatin 10mg/kg/d	Reduce infarct size-protection blocked by COX-2 and iNOS inhibitors.	Atorvastatin cardioprotection requires iNOS and COX-2.
Matsuki et al 2006[80]	In vivo rat	Fluvastatin given at 10mg/kg orally for 2 weeks before I/R or given IV just prior to ischaemia or reperfusion.	Reduce infarct size with only fluvastatin chronic treatment. Protection blocked by L-NAME.	Fluvastatin only protects when given chronically.
Penumathsa et al 2007[81]	In vivo hypercholesterolaemic rat	Oral gavage with 3 days atorvastatin 1mg/kg/d and resveratrol 20mg/kg/d	Synergistic protection associated with Akt and eNOS phosphorylation.	Synergistic protection associated with Akt eNOS phosphorylation.
Ye et al 2007[82]	Isolated rat heart	Oral gavage with 3 days subthreshold atorvastatin 2mg/kg/d and dipyridamole 6mg/kg/d	Synergistic protection associated with eNOS phosphorylation.	Synergistic protection associated with eNOS phosphorylation.
Manickavasagam et al 2007[83]	Isolated rat heart	Oral gavage with 3 days subthreshold atorvastatin 2mg/kg/d and cilostazol 20mg/kg/d	Synergistic protection associated with eNOS phosphorylation.	Synergistic protection associated with eNOS phosphorylation.
Bulhak et al 2007[84]	In vivo rat heart	Intraperitoneal injection of rosuvastatin (10mg/kg) for 2 days.	Reduced infarct size.	Rosuvastatin cardioprotection associated with inhibition of geranylgeranyl pyrophosphate and altered RhoA membrane translocation.
Birnbaum et al 2008[85]	Isolated rat heart	Oral gavage for 3 days with atorvastatin 10mg/kg/d, simvastatin 10mg/kg/d, simvastatin 2mg/kg/d, simvastatin 2mg/kg/d, + ezetimibe 1mg/kg/d.	Reduced infarct size with atorvastatin and high dose simvastatin but not low dose simvastatin or ezetimibe. Protection associated with upregulation of NOS.	Lipid-lowering drug ezetimibe is not cardioprotective.

Study	Experimental Model	Treatment regime	Key results	Novel mechanistic insight
Kocsis et al. 2008[86]	Isolated rat hearts	Oral gavage for 12 days with lovastatin 15mg/kg/d or lovastatin 50 µmol/l perfused acutely during IR.	Acute lovastatin abolished myocardial protection from IPC and failed to protect in its own right but did not affect protection from IPost. Chronic lovastatin ↓ infarct size, abolished protection from IPost but IPC remained protective. ↓ Myocardial coenzyme Q9	Chronic lovastatin is cardioprotective. Lovastatin may interfere with myocardial adaptation to ischaemic stress.
Szarszoi, 2008[87]	Isolated rat hearts	Oral chronic dosing with simvastatin 10mg/kg for 2 weeks prior to ischaemia or acutely 10 µmol/l at reperfusion.	Acute simvastatin preserved contractile force following IRI but chronic simvastatin showed no protection.	Chronic simvastatin does not protect against IRI but acute dosing does.
Thuc, 2010[63]	Cultured rat cardiomyocytes and isolated rat hearts	Cells cultured with pravastatin 10mins prior to injury with H_2O_2. Pravastatin administered 10 mins prior to ischaemia or at reperfusion in isolated hearts.	Pravastatin limited mitochondrial injury and cell death. Prior to ischaemia and at reperfusion infarct size was reduced. ERK1/2 upregulated, ROS, mitoK$_{ATP}$ and PKC implicated.	Pravastatin appeared to limit cell injury through ROS but protection is mediated via a ROS burst and RISK pathway.

Subsequent experimental studies have demonstrated pre-ischaemic cardioprotection in the absence of cholesterol-lowering using other statins including rosuvastatin[69], atorvastatin[70], pitavastatin and cerivastatin[74] and lovastatin[86]. Interestingly, lovastatin has been reported as being protective when given chronically but not when perfused acutely in isolated rat hearts. The acute regime abolished protection from IPC but IPost remained protective, whilst with chronic use lovastatin abolished protection from IPost but IPC was unaffected[86]. The authors postulate that in these scenarios lovastatin reduces coenzyme Q9 and reduces the ability of the myocardium to adapt to ischaemic stress. Simvastatin-induced cardioprotection has been

reported in other clinically relevant animal models of disease including atherosclerosis (using the ApoE -/- mice)[64] and type II diabetes (using the Db/Db mice)[65]. Many of these studies have also investigated the effect of pre-ischaemic statin therapy when administered in vivo for 1-21 days, exploring a number of different types of cardioprotective mechanisms, and therefore a direct protective effect on the myocardium or at the level of the cardiomyocyte could not be inferred. In this respect, experimental studies have made progress by demonstrating acute cardioprotective effects with statin therapy when administered 20-60 minutes before or immediately prior to acute myocardial ischaemia-reperfusion injury insult[67] [72 74]. However, other experimental studies have demonstrated cardioprotection in the absence of endothelial cells and systemic factors such as neutrophils in the isolated buffer-perfused heart as well as in cultured human ventricular cardiomyocytes subjected to simulated ischaemia-reperfusion injury[75], confirming that statin therapy is able to exert direct cardioprotective effects on the heart at the level of the cardiomyocyte.

An interesting finding by our research group concerning the pre-ischaemic cardioprotective effect elicited by atorvastatin pre-treatment in the rat heart was that chronic treatment with 2 weeks therapy failed to limit-infarct size, although the cardioprotective effect could be re-captured by administering an acute high-dose of atorvastatin immediately prior to IRI[78]. This finding was attributed to the down-regulation of the PI3K-Akt signal transduction pathway, a critical cellular protective pathway (see later section). This important finding has clinical implications for CHD patients most of whom are already on chronic statin therapy- the implication being that their current statin treatment regime may not confer cardioprotection against an episode of acute myocardial ischaemia-reperfusion injury, although the beneficial cardioprotective effect may be recaptured by administering an additional high-dose of statin immediately prior to IRI (see later section). One other study has replicated the loss of

cardioprotection with chronic dosing[87] however, it must be appreciated that other experimental studies have failed to observe a similar loss, which may be due to the use of different animal models and experimental technique (see table 1 for details)[70 71 77 80 86].

Although the above studies provide evidence that statin therapy can protect the myocardium against acute ischaemia-reperfusion injury when administered either chronically or acutely prior to IRI, there is an additional target for statin therapy which also needs to be considered. In this regard, it has been shown that statin therapy can also confer cardioprotection when administered after the onset of myocardial ischaemia and at the onset of myocardial reperfusion (see table 2).

Statin therapy administered at the onset of myocardial reperfusion

Experimental studies have demonstrated that acute statin therapy has the ability to confer cardioprotection even when administered after the onset of myocardial ischaemia and at the onset of reperfusion. Isolated working rat hearts subjected to IRI were significantly protected by the administration of simvastatin given after the onset of myocardial ischaemia and throughout the IR period[68]-see table 1. However, the first experimental study to report infarct-limitation with statin therapy administered at the onset of myocardial reperfusion was by Yellon's group in 2003[88]. In that study it was reported that atorvastatin reduced myocardial infarct size, when administered to isolated perfused murine hearts at the onset of myocardial reperfusion, demonstrating that the direct cardioprotective effects of statin therapy could be extended into the reperfusion phase. Subsequent studies have reported similar findings using simvastatin[89], mevastatin and pravastatin[63 75 90] but interestingly not with fluvastatin[80], although as the authors suggest, the optimal acute dose of fluvastatin may not have been used in their study. These experimental findings have important clinical implications for patients presenting with an acute myocardial infarction in which the

administration of statin therapy at the time of reperfusion may confer cardioprotection. The

mechanistic pathway underlying this direct cardioprotective effect elicited by statin therapy is

the subject of ongoing investigation and is reviewed in the next section.

Table 2: Experimental cardioprotection with Statin therapy given prior to myocardial reperfusion.

Study	Experimental Model	Treatment regime	Key results	Novel mechanistic insight
Post-ischaemic cardioprotection elicited by Statin therapy				
Bell et al 2003[88]	Perfused mouse heart	Atorvastatin given for the first 15 minutes of myocardial reperfusion.	Smaller infarct size. Protection associated with Akt and eNOS phosphorylation and blocked by wortmannin.	Cardioprotection with atorvastatin given at reperfusion through PI3K-Akt-eNOS pathway.
Bergmann et al 2004[90]	Isolated rat cardiomyocytes	Mevastatin and pravastatin given prior to reoxygenation. 6 hours hypoxia, 18 and 24 hours reoxygenation.	Inhibited apoptosis by both mevastatin and pravastatin.	Cardioprotection by mevastatin and pravastatin via Akt, GSK3β and inhibition of Caspase 3.
Wolfrum et al 2004[89]	In vivo rat	Activated simvastatin 1mg/kg given 3 mins prior to reperfusion	Reduced myocardial infarct size. Protection blocked by PI3K inhibitor and L-NAME.	Cardioprotection at reperfusion mediated via PI3K-Akt pathway. Reduction in infarct size after 3 and 24 hours reperfusion when simvastatin given at reperfusion via PI3k/Akt
Efthymiou et al 2005[91]	Perfused mouse heart	Atorvastatin given for the first 15 minutes of myocardial reperfusion.	Smaller infarct size. Protection blocked by pharmacological inhibition of PI3K, p38 MAPK.	Cardioprotection with atorvastatin given at reperfusion through PI3K-Akt and p38MAPK-HSP27 pathways.
Study	**Experimental Model**	**Treatment regime**	**Key results**	**Novel mechanistic insight**

Matsuki et al 2006[80]	In vivo rat heart	Fluvastatin given iv at reperfusion.	No protection at reperfusion.	Fluvastatin does not protect when given at reperfusion.
Shakkottai et al 2008[92]	Isolated rat heart	Atorvastatin given just prior to reperfusion.	Reduced infarct size and protection not blocked by 8-SPT.	Cardioprotective action of atorvastatin at reperfusion not mediated through adenosine receptor.
D'Annunzio et al 2009[93]	Isolated rabbit heart	Rosuvastatin given at reperfusion (30mins I, 120mins R)	Reduced infarct size in normal and hypercholesterolaemic rabbits. Attenuated MMP-2 action.	Rosuvastatin is cardioprotective with MMP-2 implicated in the mechanism.

Potential mechanisms underlying statin-induced cardioprotection

Nitric oxide has been implicated as a crucial signalling molecule in the mechanism of cardioprotection and the other pleiotropic effects of statin therapy (figure 3). Experimental studies have demonstrated that statins stabilise eNOS mRNA, upregulate and activate eNOS and increase NO production[61 65 94]. The mechanism through which statins actually activate the nitric oxide signalling pathway is still the subject of investigation but it may be through the PI3K-Akt-eNOS pathway[88] (see later).

By inhibiting the formation of mevalonate and thus the isoprenoids[59], critical proteins in the inflammatory cascade are inhibited (in particular Ras, Rho and Rab) which act via nuclear factor-κB and, as such, statins exhibit a powerful anti-inflammatory effect[95]. By inhibiting isoprenylation and preventing the formation of geranylgeranyl pyrophosphate (see figure 2), rosuvastatin has been shown to prevent Rho A translocating to the plasma membrane, increasing the cytosol-to-membrane ratio in the myocardium and providing infarct size reduction. The addition of GGPP abrogates this protection[84]. The inhibition of Rho also prevents its inhibitory action on NO and thus provides a further mechanism of NO

upregulation[96]. Furthermore, inhibition of Rho kinase activates PI3k/Akt, providing cardioprotection- see later[97][98] .

Alternatively, Giricz and colleagues[99] have reported that in fact lovastatin, and by inference all statin medications, do not increase cardiac NO levels. In a hyperlipidaemic rat heart model, the authors postulate that increased reactive oxygen species production reduces NO availability. The authors attempted to restore cardiac NO availability by inhibiting the mevalonate pathway with lovastatin or by cholesterol rich diet but were unable. Giving farnesyl, a major metabolite in the mevalonate pathway, did not alter NO levels either. However the authors were unable to conclusively demonstrate that by using lovastatin or a cholesterol rich diet they had completely blocked the mevalonate pathway and the hearts were not subjected to any ischaemia or reperfusion stimulus. The authors have previously found that treatment with farnesyl restores pacing preconditioning in a permanent coronary occlusion hyperlipidaemic rat model although not by restoring cardiac NO synthesis[100].

Figure 3: Summary of the important pleiotropic effects of Statins (please see text for detail and an explanation of the abbreviations).
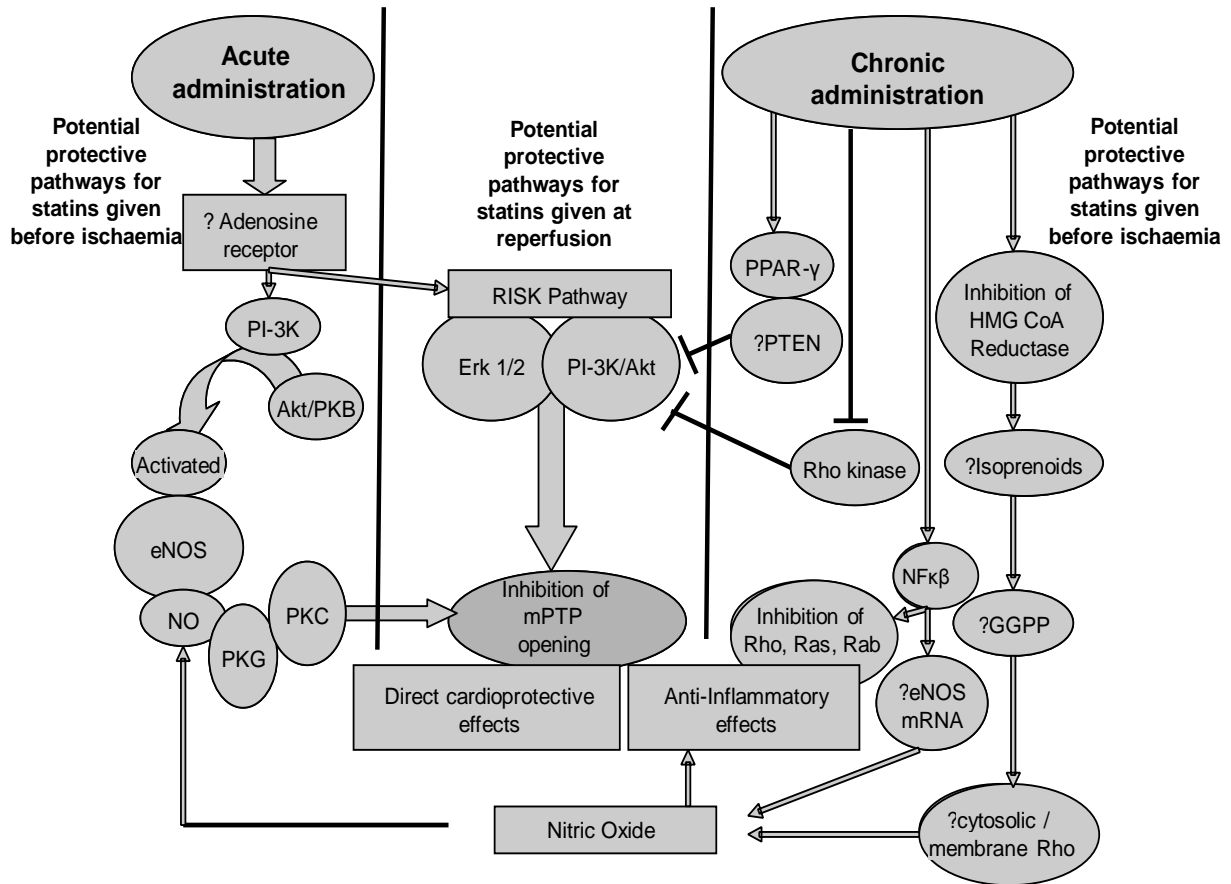
Statins and the Reperfusion Injury Salvage Kinase (RISK) pathway

Subsequent experimental studies from Yellon's group suggested that the direct cardioprotective effect elicited by Statin therapy was due to the activation of the Reperfusion Injury Salvage Kinase (RISK) pathway, the term given to a group of pro-survival kinases, the activation of which at the onset of myocardial reperfusion confers powerful cardioprotection in the animal heart[46][53]. In that study, it was demonstrated that atorvastatin administered at the onset of myocardial reperfusion reduced myocardial infarct size by 50% in isolated perfused murine hearts, and that this protective effect was mediated through the activation of the PI3K-Akt-eNOS signal transduction pathway, given that the infarct-limiting effects were abrogated in the presence of a PI3K inhibitor and were lost in mice lacking eNOS[88]. In addition, the p38MAPK-HSP27 signalling pathway has also been implicated in the cardioprotection elicited by atorvastatin if given at the onset of reperfusion[91]. Subsequent studies have also implicated the recruitment of the RISK pathway in cardioprotection obtained with statin pre-treatment of hearts[74]. The myocardial protective phenomena of pre- and postconditioning appear to activate the RISK pathway at reperfusion possibly via the adenosine receptor; preconditioning being mediated via the A1[101], A2a[102] and A3 forms[103] and post-conditioning mediated via the A2b form[104]. The mechanism through which elements of the RISK pathway are actually activated by statin therapy is however unclear, although it may be through the potentiation of myocardial adenosine by ecto-5'-nucleotidase[74]. Interestingly the non-selective adenosine receptor blocker, 8-sulfophenyl theophylline, was reported to blunt the infarct-limitation conferred by statin pre-treatment in dogs[74] and both theophylline and 8-SPT have been demonstrated to prevent atorvastatin-induced Akt, ERK 1/2, and eNOS phosphorylation[105]. Recently the blockade of adenosine receptor binding by caffeine, in a rat model of IRI, has been reported to prevent the cardioprotection afforded by

atorvastatin[106], although in a recent study from our group we were unable to abolish atorvastatin-induced infarct-limitation at the onset of reperfusion in the perfused rat heart using SPT as a pharmacological inhibitor of the adenosine receptor[92]. Our study was in an isolated rat heart model with atorvastatin given at the onset of reperfusion where as Ye et al. used an in-vivo rat model with 3 days of oral dosing of both atorvastatin and caffeine; this may explain the discrepancy between the results seen. Nonetheless the RISK pathway appears to be a core component of a common cardioprotective pathway which converges on and inhibits the opening of the mitochondrial permeability transition pore, thereby preventing cardiomyocyte death[51] [54]. As mentioned earlier the waning of the cardioprotective effect of atorvastatin given chronically for 2 weeks observed by our group is thought to be related to the upregulation of phosphatase and tensin homolog deleted on chromosome ten (PTEN), a phosphatase which down-regulates the PI3K-Akt component of the RISK pathway[78] and prevents the detrimental effects of prolonged upregulation of this growth promoting pathway. The additional higher dose of atorvastatin is presumed to restore cardioprotection by re-activating the PI3K-Akt pathway[78].

Clinical Cardioprotection using Statin Therapy

The experimental animal studies suggest that statin therapy has the ability to protect the myocardium against the detrimental effects of acute ischaemia-reperfusion injury irrespective of whether it is administered prior to the index ischaemic event or at the onset of myocardial reperfusion. As such it could be argued that statin therapy may theoretically confer cardioprotection in a range of clinical settings including those in which the index ischaemic event can be readily anticipated such as planned cardiac surgery or elective percutaneous coronary intervention (PCI), and in those settings in which myocardial ischaemia has already

commenced such as in acute myocardial infarction patients undergoing myocardial reperfusion using thrombolytic therapy or primary PCI.

Statin therapy and percutaneous coronary intervention

Peri-operative myocardial necrosis following elective or urgent percutaneous coronary intervention (PCI) has been documented using serum cardiac enzymes and cardiac magnetic resonance imaging[107] and its presence has been linked to clinical outcomes[108-110]. In recently published guidelines, the myocardial infarction associated with PCI (termed type 4a), has been defined as an increase in a biomarker (preferably Troponin) of more than three times the 99th percentile upper reference limit[111]. The aetiology of myocardial injury during PCI is likely to be multi-factorial with individual patient's anatomy, complexity of procedure, and concurrent medications playing a role[112]. Clinical studies suggest that Statin therapy may be beneficial in the setting of PCI in terms of reducing peri-procedural myocardial injury and potentially improving clinical outcomes.

With respect to elective PCI, initial studies were designed to investigate the potential protective endothelial effects of statin therapy in terms of coronary artery lumen diameter and restenosis rates following PCI, with the statin generally initiated following PCI. In a retrospective analysis of 5052 patients undergoing elective PCI, Chan and colleagues[113] were the first to demonstrate an association between statin use prior to PCI with improved clinical outcomes in terms of fewer peri-procedural MI's (defined by CKMB 3x upper limit of normal, 5.7% versus 8.1%, P=0.038), and a mortality reduction at 30 days (0.8% versus 1.5%; hazard ratio, 0.53; P=0.048) and at 6 months (2.4% versus 3.6%;hazard ratio, 0.67; P=0.046)[113][114] (see table 3).

Table 3: Statin therapy and percutaneous coronary intervention

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Elective PCI					
Bertrand, 1997[115]	Multi centre, randomised, placebo controlled	695 patients having undergone successful balloon angioplasty (no stent)	Pravastatin 40mg/d or placebo started after coronary intervention	6 months	No difference in luminal diameter or rate of restenosis. No difference in clinical outcomes.
Serruys, 1999[116]	Randomised, placebo controlled, double blind	1054 patients undergoing elective coronary artery balloon angioplasty (no stent)	40mg twice/d fluvastatin or placebo started 2-3 weeks pre-PTCA	Angiographic restenosis at 26 weeks and MACE outcomes at 40 weeks.	No effect on restenosis. Death and MI reduced from 4 to 1.4% (log rank P=0.025)
Schartl, 2001[117]	Open label, randomised, multi centre	131 patients following PCI	Atorvastatin or usual care. Atorvastatin titrated to achieve LDL-C <100mg/dl.	Plaque volume at 12 months	A non-significant reduction in plaque volume. No difference in MACE rates.
Serruys, 2002[118]	Randomised, placebo controlled, double blind	1677 patients who had undergone first successful PCI	80mg/d fluvastatin or placebo started at hospital discharge	Survival time free from MACE at a median follow up of 3.9 years.	Significant reduction in MACE rates in fluvastatin group. RR 0.78 (95% CI 0.64 to 0.95, P=0.01)
Pasceri, 2004[119]	Randomised, placebo controlled, double blind	153 Statin naive patients undergoing elective PCI	40mg/d atorvastatin or placebo started 7 days prior to procedure.	Post procedural peak levels of CK-MB, Trop-I and Myoglobin.	Atorvastatin significantly ↓ myocardial enzyme release post elective PCI.
Brigouri, 2004[120]	Randomised	451 patients due to undergo elective PCI	Randomised to Statin (variable type) or no Statin	Incidence of large peri-procedural myocardial injury (CK-MB and Trop-I ↑ ≥5 times ULN) at 6 and 12 hours post PCI	↓incidence of CK-MB release 5x ULN, and ↓ incidence of Trop I 5x ULN (32 to 23.5%, p=0.043).

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Mood, 2007[121]	Meta-analysis	3941 patients undergoing elective PCI	Randomised to Statin (variable type) started periprocedure	Clinical outcomes from 1 day to 45 months	↓ incidence of myocardial infarction post PCI (0.57, 95%CI 0.42 to 0.78, p=0.0001)
Naples II, 2009[122]	Randomised, placebo controlled, double blind	668 statin naive patients undergoing elective PCI	Randomised to 80mg atorvastatin loading 24hours prior to PCI or placebo	Incidence of CK-MB and Trop-T >3 times ULN at 6 and 12 hours post-PCI	↓ CK-MB (9.5% vs 15.8%, 95% CI 0.35-0.89, p = 0.01). ↓ Trop T 26.6% vs 39.1%, 95% CI 0.40-0.78, p < 0.001)
Armyda-Recapture, 2009[123]	Randomised, placebo controlled, double blind	352 patients established on statins undergoing elective PCI	Randomised to 80mg 'reload' 12 hours pre-PCI and 40mg immediately pre-PCI	MACE at 30 days. Peri-procedural cardiac enzyme release. Peak post procedure CRP.	↓ MACE 3.4% vs. 9.1%, p = 0.04. ↓ incidence of CKMB and Trop-T >3xULN, 13% vs. 23%, p = 0.02 and 36% vs. 47%, p = 0.03.
Veselka, 2009[124]	Randomised, controlled, open label	200 statin naive patients undergoing elective PCI	Randomised to 80mg atorvastatin daily for 2 days prior to PCI or no statin	Cardiac enzyme release between 16 and 24hours post PCI	No difference in CK-MB or TnI release.
Cay, 2010[125]	Randomised, open label, controlled.	299 statin naive patients undergoing elective PCI	Randomised to 40mg rosuvastatin 24 hrs pre-PCI or no treatment.	CK-MB and TnI at 12hrs post PCI	↓CK-MB >3× ULN: 0.7% vs. 11.0%. ↓TnI >3× ULN 10.5% vs 39.0%. Any CK-MB↑ >ULN 10.5% vs 34.2%. Any cTnI↑ >ULN 20.9% vs 61.6%, p<0.001 for all.
Urgent PCI					
Chang, 2004[126]	Observational	119 patients undergoing urgent PCI following ACS	Statins or no Statin therapy	Myocardial injury measured by CK-MB or CK ≥ 3 times ULN. MACE over 6 months	↓ Myocardial injury 10 to 2% (p=0.04). ↓MACE 21 to 17% (p=0.015)

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Chyrchel, 2006[127]	Randomised, open label	140 patients undergoing urgent PCI following ACS	80mg atorvastatin daily started 3 days prior to PCI and then 40mg daily or just 40mg daily started after PCI	MACE rates over approx. 600 days	↓composite endpoint of death, MI & re-PCI rates from 25.9 to 8.1% (p=0.006)
Patti, 2007[128]	Multi centre, randomised, placebo controlled, double blind	171 patients undergoing urgent PCI following ACS	80mg atorvastatin 12 hours prior to PCI with 40mg just prior and continuing or placebo until after PCI and then atorvastatin 40mg started	30 day MACE rates. Peri-procedural myocardial injury defined by twice the ULN of CK-MB, Trop I or a 2 fold increase.	↓MACE (OR 0.12, 95%CI 0.05 to 0.5, p=0.004). ↓incidence of CK-MB release 7% v 27% (p=0.001), ↓ incidence of Trop I release 41% v 58% (p=0.039).
Gibson, 2009[129]	Post hoc analysis of the PROVE-IT[130] study	2868 patients who underwent urgent PCI for ACS	80mg atorvastatin or 40mg pravastatin	2 year MACE rate	↓MACE (21.5% atorvastatin vs 26.5% pravastatin, (HR: 0.78, 95%CI: 0.67 to 0.91, p=0.001). ↓target vessel revascularisation.
Elective and urgent PCI					
Chan, 2002[113]	Observational	5052 patients undergoing elective and urgent PCI (recent MI excluded)	26.5% of patients taking variable Statins pre-procedure.	Mortality at 30 days and 6 months.	↓unadjusted 30 mortality from 1.5 to 0.8% (HR 0.53, log rank P=0.048). ↓unadjusted 6 months mortality from 3.6 to 2.4% (HR 0.67, log rank P=0.046)
Chan, 2003[114]	Observational	1552 patients undergoing elective or urgent PCI	39.6% of patients taking variable Statins pre-procedure.	Level of hsCRP and peri-procedural MI (defined as CKMB >3 times ULN)	Statin use associated with ↓hsCRP (0.4 v 0.5, p=0.012). Statins ↓ peri-procedural MI (5.7 v 8.1% p=0.038)

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Merla, 2007[130]	Meta-analysis	4751 patients undergoing all types of PCI	Observational and randomised studies (variable Statins) started periprocedure.	Incidence of peri-procedural myonecrosis	Incidence of myonecrosis reduced from 17.5% to 9% in the Statin group. (OR 0.45, 95% CI 0.33 to 0.62, p<0.01)

The Atorvastatin for the Reduction of MYocardial Damage during Angioplasty (ARMYDA) study[119] was one of the first prospective randomised studies to investigate whether acute statin therapy could limit peri-procedural myocardial injury in statin-naïve patients- 153 patients undergoing elective PCI were randomised to receive either 40 mg/day atorvastatin or placebo for 7 days prior to the PCI. Its primary outcome measure was peri-procedural myocardial infarction, defined as a CK-MB> 2 times the upper limit of normal post-PCI. One week of atorvastatin treatment prior to PCI reduced the incidence of peri-procedural MI from 18% to 5% (P=0.025). Brigouri and colleagues[120] also examined the effect of statin therapy started prior to PCI on the incidence of peri-procedural myocardial infarction- 451 patients undergoing elective PCI were randomised to receive a statin or no treatment. The choice of statin was left to the discretion of the physician (atorvastatin 29%, pravastatin 29%, simvastatin 39% and fluvastatin 3%) and was started at least 3 days prior to the procedure. Statin therapy was reported to reduce peri-procedural MI (defined as 5 times the ULN of either CK-MB or Troponin I) when compared to no treatment with respect to CK-MB (8 with statin versus 15.6 in control, P=0.012) and Troponin-I (23.5 with statin versus 32 in control, P=0.043). Brigouri's group have recently followed this study with Naples II which randomised 668 statin naive patients to an 80mg loading dose of atorvastatin within 24 hours of elective PCI. The atorvastatin treated group were reported to have a significantly reduced

incidence of CK-MB and Troponin-T release greater than 3 times the ULN (CK-MB: 9.5% vs. 15.8%, 95% confidence interval 0.35-0.89, odds ratio 0.56, p = 0.01) (Trop T: 26.6% vs. 39.1%, 95% CI 0.40-0.78, OR 0.56, p < 0.001).[122]

A meta-analysis undertaken by Birnbaum's group[130] demonstrated that statin therapy prior to elective PCI reduced the incidence of myocardial necrosis, but the number of studies analysed was small. Furthermore, a meta-analysis by Mood et al.[121] of statin use prior to elective PCI and their effect on outcome, included studies totalling 3941 patients and showed a reduction of peri-procedural MI of 43% in those taking statin therapy. The reduction in cardiovascular events occurred early post-procedure and was sustained, with an average follow up of 22.4 months. There was however no significant reduction in all cause or cardiovascular mortality. The authors acknowledged that their analysis was limited in some measure due to variation in study design and the necessity of comparing sometimes heterogeneous treatment protocols. A large proportion of the positive effect observed in this meta-analysis was driven by the ARMYDA study[119] which included peri-procedural myocardial infarction in the figures of longer-term adverse cardiac events. Based on the hypothesis put forward by our group, with respect to the fact that the cardioprotective benefits of statin therapy may wane over time but can be recaptured by a further acute dose[78], the ARMYDA investigators have also performed ARMYDA-RECAPTURE to determine whether patients already on chronic standard statin therapy due to undergo PCI will incur further benefit from an acute dose of statin therapy prior to PCI. 383 patients already established on statin medication scheduled to undergo PCI were randomised to a 'reload' of 80mg atorvastatin 12 hours pre-procedure and 40mg immediately pre-PCI. This was a mixed group with 47% of patients having recently experienced an acute coronary syndrome. The incidence of 30 day MACE was significantly lower in patients treated with atorvastatin

reload prior to PCI (3.7% vs. 9.4%, p = 0.037), however this was entirely driven by a reduction in periprocedural myocardial infarction in the acute coronary syndrome patients[123] suggesting perhaps that patients undergoing low risk elective PCI may have little to gain. In this regard a further study in 200 patients has shown no benefit in elective PCI of 48hours pretreatment with 80mg atorvastatin and although this study is relatively small and unblinded it does mean that a conclusive benefit in elective PCI is yet to be demonstrated[124].

With respect to urgent PCI, a few studies have investigated statins as cardioprotective agents exclusively in patients presenting with an acute coronary syndrome undergoing PCI (see table 3). An initial observational study of 119 patients by Chang et al[126] suggested that patients who were undergoing PCI following an acute coronary syndrome and were already taking a statin medication had a reduced incidence of cardiac events compared to those without statins. The incidence of myonecrosis (defined by a peak CK-MB or CK 3 times the ULN was reduced from 10% to 2% (P=0.04) and adverse cardiac events over 6 months of follow up were reduced from 21% to 17% (P=0.015) in those taking statins.

Two prospective clinical studies have investigated the effect of statin therapy in patients with non-ST elevation MI (NSTEMI) undergoing PCI. Chyrchel and colleagues[127] randomised 140 patients with NSTEMI who were undergoing an early invasive PCI strategy to receive either 80mg of atorvastatin starting 3 days prior to intervention and then 40mg daily or no treatment prior to PCI but 40mg of atorvastatin daily commencing after the PCI. The patients were followed over approximately 600 days with an analysis of MACE as the primary outcome. Strikingly, the early initiation of atorvastatin reduced the composite end point of death, MI or repeat PCI from 25.9% to 8.1% (P=0.006). The authors did not define on what criteria they diagnosed the end point of myocardial infarction and did not describe a distinction between procedure related myonecrosis and later cardiac events. However these

were very promising results suggesting that by starting statin therapy just 3 days earlier in these patients it is possible to make a significant impact on long term outcomes.

In ARMYDA-ACS[128], 171 NSTEMI patients were randomised to receive 80 mg of atorvastatin 12 hours prior to the procedure and an additional 40mg, 2 hours prior to the procedure or placebo (all patients received 40mg atorvastatin daily following intervention regardless of initial randomisation). They reported a reduction in 30 day MACE from 17% in the placebo group to 5% in the atorvastatin group (p=0.01), although this was due to a reduction in peri-procedural myocardial necrosis as there were no cardiac events in either group after day 2 post-PCI. A subsequent sub-group analysis has suggested that reduced levels of circulating adhesion molecules (ICAM1 and VCAM1) may be partly responsible[131]. A retrospective analysis of the PROVE-IT data was conducted to explore the effects of atorvastatin 80mg versus pravastatin 40mg in those patients with ACS who had just undergone PCI prior to recruitment[129]. 2848 patients fulfilled this criteria and follow up over a median of 2 years revealed a 22% relative risk reduction in MACE rate (26.5 vs 21.5%, p=0.001) in the atorvastatin group. This included a reduction in the composite outcome of death and MI and a reduction in target vessel revascularisation. This is of course a post-hoc analysis of a tightly controlled study population and with PCI conducted in the bare metal stent era and so results should be interpreted with some caution, however this may be a hint at improved longer term outcomes with intensive over moderate statin use in PCI following ACS.

In summary, statin therapy appears to limit peri-procedural myocardial necrosis and subsequent non-fatal events when started a short duration before both elective and urgent PCI but at the present time it is not conclusive whether this impacts on longer term mortality. The majority of patients undergoing elective PCI would be expected to be on long-standing statin

therapy, although patients undergoing a diagnostic coronary angiogram query proceed to PCI or those with acute coronary syndromes undergoing urgent PCI, may not be. The weight of benefit at present would appear to be in those patients with an ACS undergoing PCI.

<u>Statin therapy in acute coronary syndromes</u>

The majority of clinical studies investigating the effects of statin therapy in patients presenting with an acute coronary syndrome have focused on the initiation of statin therapy 1-2 days following admission. Large randomised placebo controlled studies have already established chronic statin use as effective medical therapy for the primary and secondary prevention of cardiovascular events. Subsequent studies have begun to investigate the effects of acute statin therapy on patients presenting with acute coronary syndromes, but in the majority of these studies statin therapy was initiated 24 hours after the ACS (see table 4).

Table 4: Statin therapy in acute coronary syndromes

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Arntz, 2000[132]	Prospective, open label, randomised	135 patients undergoing PCI following new Q wave infarction or ACS	Pravastatin started at 20mg/d and titrated for LDL-C <130mg/dl or standard care by family physician	Angiographic minimal luminal diameter and MACE rates at 6 and 24 months	↓rate of progression of coronary atherosclerosis. ↓MACE rates, OR 3.6, 95%CI 1.6 to 7.8, p=0.005)
Kayikcioglu, 2002[133]	Randomised, placebo controlled	164 patients having been thrombolysed for acute MI	Pravastatin 40mg or placebo	Clinical outcomes at 6 months	↓rate of subsequent angina, 59.5% to 30% (p=0.018) , ↓composite MACE, 75.6% to 32.5% (p=0.0001).

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Okazaki, 2004[134]	Randomised, open label	70 patients following an ACS and PCI	20mg atorvastatin or control (lipid lowering diet and cholesterol absorption inhibitor)	Atherosclerotic plaque volume at 6 months, MACE rates.	Significant reduction in plaque volume. No difference in MACE rates initially but extended recruitment subsequently showed a benefit[136].
Cannon, 2004[135]	Multi-centre, randomised, double blind, placebo controlled	4162 patients following ACS	Atorvastatin 80mg aiming LDL-C<70mg/dl or pravastatin 40mg aiming LDL-C<100mg/dl	MACE rates over average 2 year follow up.	Relative risk reduction in composite MACE of 16% (95% CI 5 to 26%, p=0.005) in atorvastatin group
De Lemos, 2004[136]	Multi-centre, randomised, double blind, placebo controlled	4497 patients	Simvastatin 40mg and then 80mg or placebo for one month and then simvastatin 40mg	MACE rates over 4 months and 2 years.	No reduction in MACE at 4 months. At 2 years rate of cardiovascular death ↓from 5.4 to 4.1% (p=0.05).
Colivicchi, 2002[137]	Prospective, randomised	81 patients following ACS not amenable to revascularisation	Atorvastatin 80mg or conventional treatment	12 month MACE rates	↓MACE from 46 to 22% (OR 0.33, 95%CI 0.12 to 0.88, P=0.0025)
Schwartz, 2001[138]	Multi-centre, randomised, double blind, placebo controlled	3086 patients following an ACS	Atorvastatin 80mg or placebo to start 24 to 96 hours after admission	MACE rates over 16weeks	↓recurrent ischaemia requiring hospitalisation from 8.4 to 6.2% (P=0.02). No difference in other MACE outcomes.
Liem, 2002[139]	Randomised, placebo controlled, double blind	540 patients following an ACS	Fluvastatin 80mg daily started within 2 weeks of event	Observed ischaemia and MACE rates over 12 months	No difference in observed ischaemia, no difference in MACE

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Thompson, 2004[140]	Randomised, placebo controlled, double blind	3408 patients following an ACS	Pravastatin 20 to 40mg or placebo	MACE rates at 30 days	Non-significant trend to benefit of pravastatin.
Lenderink, 2006[141]	Retrospective cohort	10,484 patients following an ACS	Statin naive survivors at 24 hours receiving Statins versus those not.	All cause mortality at 7 and 30 days.	↓7 day mortality (0.4%v2.6%, unadjusted HR 0.16, 95% CI 0.08 to 0.37).Non-significant ↓mortality at 30days.
Briel, 2006[142]	Meta-analysis of randomised controlled trials	13,024 patients following an ACS	Variety of Statins and doses started within 14 days	MACE outcomes at 1 and 4 months	No reduction in death, MI or CVA up to 4 months.
Aronson, 2008[143]	Prospective observational study	1,563 patients following acute MI	Statin started in-hospital pre-discharge (type and dose not recorded) or not	Admission with heart failure over median follow up of 17 months.	Pre-discharge statin therapy ↓ admission with heart failure from 14.8 to 6.5% (p<0.0001).
Kim, 2010[144]	Prospective, single blind, randomised controlled study	171 patients with STEMI	80mg or 10mg atorvastatin pre-PPCI and then 10mg long term	30 day MACE rate	No reduction in MACE rate. Improved TIMI frame count, MBG and ST segment resolution.

Initial retrospective observational studies suggested that the early initiation of statin therapy may well provide a reduction in short term cardiovascular adverse event[145-148] although not all studies supported these findings[149]. Accordingly, small randomised clinical studies were performed[132-134 150] (often with mixed end-points), which overall suggested that the early initiation of a statin was beneficial.

Larger clinical studies that were designed to investigate early statin therapy in the setting of ACS have been undertaken. In the PROVE-IT-TIMI 22 clinical study[135] 4162 patients within

10 days of presentation with an ACS were enrolled and randomised to receive either 80mg of atorvastatin/day or 40mg pravastatin/day. The higher-dose atorvastatin therapy was found to reduce the relative risk at 2 years of the composite endpoint (death, MI, ACS requiring hospitalisation, coronary revascularisation or stroke) by 16% (95% CI 5 to 26%, p=0.005). Following this the much awaited Z phase of the A to Z study[136] did not live up to the expectation placed upon it by the positive findings from PROVE-IT. The investigators randomised 4497 patients who had presented with an ACS to one month of simvastatin 40mg followed by 80mg continuing or placebo for 4 months followed by continuing on 20mg. Results at 4 months surprisingly failed to show any difference in the primary end point of composite MACE. Over the total of 2 years follow up there was a benefit from the more intensive, earlier initiated regime with a modest non-significant reduction in all cause mortality and cardiovascular death from 6.7 to 5.5% (p=0.08) and from 5.4 to 4.1% (p=0.05) respectively. It appeared that PROVE-IT and A to Z had generated disparate results, and indeed A to Z not exhibiting earlier benefits appeared to contradict the growing body of evidence from the smaller studies described above[132-134 150].

A posthoc comparison[151] of the studies suggests that the differences in outcome are explained by slightly different cohort characteristics (in terms of baseline inflammatory status, LDL-cholesterol levels, intensity of statin therapy) and by a higher rate of pre-randomisation revascularisation in the PROVE-IT study. The overall conclusion of the comparison, however remains that early initiation of statin therapy with or without appropriate revascularisation following an admission with acute coronary syndrome brings a mortality benefit. Patients not amenable to any revascularisation procedure also benefit from the early use of statins[137].

The Myocardial Ischaemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study[138] was designed specifically to look at even earlier treatment with intensive atorvastatin

therapy (80mg) following an acute coronary syndrome (unstable angina or non-Q wave MI) versus placebo with therapy initiated 24 to 96 hours following admission. 3086 patients were randomised and followed over 16 weeks. There were no significant differences between the groups of death, non-fatal MI or cardiac arrest but there was a significant reduction in repeat admissions for ischaemic events in the 16 weeks of follow up (8.4% to 6.2%, p=0.02). This study showed that the beneficial effects of statins are seen very early on and as such are unlikely to be completely related to LDL-cholesterol concentration and the pleiotropic effects exert actions very soon following initiation. The FLuvastatin On Risk Diminishment following Acute myocardial infarction (FLORIDA) group undertook a randomised placebo controlled study to look at the incidence of ischaemia following the initiation of fluvastatin within 14 days following an ACS[139] in 540 patients. It did not corroborate the findings of MIRACL and no significant difference was seen between the fluvastatin and placebo groups in terms of MACE or recorded ischaemia (on ambulatory ECG). However, the outcome measures were not standard and the overall power of the study was significantly less than the initial calculations due to the loss of un-interpretable ECG's. Overall this study did not strengthen the argument for the use of statins post ACS although perhaps the difference between FLORIDA and MIRACL is that the earlier the statin can be initiated the greater the potential for benefit can be.

Further studies to clarify these issues have become more difficult as justifying placebo control is ethically difficult. The PACT study[140] attempted to explore whether pravastatin given within 24 hours of presenting with an ACS would improve 30 day outcomes. The trial was halted having recruited 3408 patients (having aimed for ten thousand) as the population of patients being screened were increasingly already on statin therapy. They showed a non-significant but favourable trend in the pravastatin group towards a 6.4% relative risk

reduction of death, recurrence of MI or readmission to hospital with unstable angina. Lenderink and colleagues[141] reported from the Euro Heart Survey (10,484 patients) on the use of statins in the very early period following presentation with an acute coronary syndrome in statin-naive patients. Those patients surviving to 24 hours who received statins had an absolute reduction in mortality at 7 days of 2.2% (unadjusted HR 0.16, 95%CI 0.08-0.37) which was maintained at 30 days but was no longer statistically significant. When separated by presentation, with ST elevation MI (STEMI) versus non-ST elevation MI (NSTEMI), statistical significance was seen in those presenting with STEMI (HR 0.17, 95% CI 0.04 to 0.7) but not NSTEMI. Although observational data, these results suggested that very early statin use, even in the setting of acute ST-elevation MI, could make a significant difference. Accordingly, Kim et al.[144] conducted the Statin STEMI study which recruited 171 patients and randomised to 80mg or 10mg atorvastatin prior to PPCI for STEMI and then 10mg atorvastatin following this. There was no difference in the primary end-point of 30 day MACE rate but they demonstrated an improvement in secondary end-points of myocardial blush grade (2.2 +/- 0.8 vs. 1.9 +/- 0.8, p = 0.02), TIMI frame count (26.9 +/- 12.3 vs. 34.1 +/- 19.0, p = 0.01) and ST segment resolution (61.8 +/- 26.2 vs. 50.6 +/- 25.8%, p = 0.01).

A thorough meta-analysis in this area concluded that on current evidence early initiation of statin therapy following an ACS is not associated with a relevant reduction in death, MI or stroke in the first 4 months following an ACS[142]. The authors did conclude, as others have done[151], that early initiation is unlikely to be harmful, may reduce the recurrence of unstable angina requiring hospitalisation in the first 4 months, may reduce the incidence of heart failure[143] and is likely to increase patient adherence to statin therapy in the longer term[152]. Current guidelines do not yet advocate the use of 'acute statins' but both the ESC and the AHA/ACC guidelines on the management of patients with NSTEMI or unstable angina

recommend starting a statin medication in all patients in the absence of contraindications and that this should be started prior to hospital discharge[153] [154]. We would argue that statins should be given as early as possible in the treatment of patients with ACS on the basis that the likelihood for harm is low and that studies appear to show benefits with early administration. The overall risk of a statin related adverse event and particularly severe myotoxicity, even with intensive therapy, is low but is significantly increased above lower doses of statins[155] and therefore the 'optimal' medical therapy is likely to vary as to the risk stratification of the patient.

There is certainly a need for further studies examining the acute administration of statins in the treatment of a suspected acute coronary syndrome which would help clarify further the optimal dosing regimen and timing. In this regard, there are clinical studies underway examining the effects of Atorvastatin therapy administered in STEMI patients undergoing primary PCI (ARMYDA-MI)[156].

Statin therapy and cardiac surgery

Cardiac surgery requiring cardiopulmonary bypass subjects the myocardium to significant global ischaemia-reperfusion injury, which can be detected as myocardial necrosis and a rise in serum cardiac enzymes, a finding which has been associated with short, medium and long-term clinical outcomes[157-159]. The mean 30 day mortality for elective CABG surgery in the UK is 1.8% but as surgery is performed in increasingly high risk groups mortality estimates can reach 10-20%[160]. The nature of the myocardial injury is not entirely clear and is likely to be multi-factorial and significantly influenced by the length of cardioplegic arrest, the composition of cardioplegic solutions, the number of grafts, the time required for each anastomosis, atheroembolisation, direct injury due to handling and retraction of the heart, systemic inflammation and the use of intracoronary shunts[161] [162]. Ischaemia-reperfusion

injury is probably the most important mode of injury, related to the global ischaemia induced by aortic cross-clamping during the performance of the distal coronary anastomoses. The importance of myocardial injury during CABG surgery has been recognised and is classified as a Type 5 Myocardial Infarction when there is evidence of increased biomarkers greater than 5 times the 99th percentile of the upper reference limit plus either new pathological Q waves or new left bundle branch block on ECG, angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium[111]. Clinical outcomes in this group are clearly worsened, and so the prior application of a cardioprotective strategy may limit the myocardial damage incurred by the PMI. The use of statins has been explored in order to try and reduce myocardial injury and improve outcomes (see table 5).

Table 5: Statin therapy and cardiac surgery

Study	Design	Type of patients (No)	Intervention	Follow up	Outcome
Pan, 2004[163]	Retrospective cohort	1663 patients undergoing primary CABG	Pre-operative Statin or not.	30 days	↓ all cause mortality from 3.75% to 1.8% (p<0.05). ↓ all cause mortality and stroke from 7.1% to 4.6% (p<0.05).
Ali, 2005[164]	Retrospective cohort (with matched propensity model analysis)	5469 patients undergoing primary CABG	Pre-operative Statin or not.	In-hospital outcome	No significant difference between matched groups in mortality or other measures.
Collard, 2006[165]	Pre-specified subset analysis of a prospective longitudinal study	2666 patients undergoing primary elective CABG	Pre-operative Statin or not.	Hospital stay	↓cardiac mortality in first 3 post-operative days (0.3% v 1.4%, p=0.03). No reduction of in-hospital MI.
Chello, 2007[166]	Randomised, placebo controlled	30 patients undergoing on-pump CABG	Simvastatin 40mg or placebo started 3 weeks pre-op	N/A	↓ IL-6 and IL-8. ↑ neutrophil apoptosis
Study	**Design**	**Type of patients (No)**	**Intervention**	**Follow up**	**Outcome**

Fedoruk, 2008[167]	Retrospective cohort	447 patients undergoing isolated cardiac valve surgery	Pre-operative Statin or not	30 days	Adjusting for risk factors- composite outcome of 30day mortality/stroke/renal failure ↓with OR 2.7 (95% CI 1.24-5.66, P=0.012)
Liakopoulos, 2008[168]	Meta-analysis	30,000+ patients undergoing cardiac surgery.	Variable types of preoperative Statin or not.	Variable	A 1.5% absolute reduction in early all cause mortality. Reduced peri-operative stroke and AF. No reduction in peri-operative MI or renal failure.
Mannacio, 2008[169]	Randomised, placebo controlled	200 patients undergoing elective CABG surgery	7 days of 20mg rosuvastatin or placebo pre-op.	8 days	↓ Trop I (peak 0.16 ± 0.15 vs 0.32 ± 0.26 ng/ml, P=0.0008), Myoglobin, CK-MB and hsCRP in postoperative period.

Initially, a retrospective study by Pan and colleagues[163] comprising 1663 patients undergoing elective CABG surgery compared those taking regular statin therapy (943 patients) against those not (720 patients) and showed a significant reduction in the 30 day post-operative all cause mortality from 3.75% to 1.8% (p=0.01) but did not show any difference in post-operative myocardial infarction, incidence of arrhythmia, occurrence of stroke or renal dysfunction. A further retrospective study by Ali and Buth[164] comprising 5469 patients undergoing CABG surgery did not report any significant benefit in those taking statins pre-operatively. Collard and colleagues[165] undertook the Multicenter Study of Peri-operative Ischemia (McSPI) Epidemiology II Study, a prospective longitudinal study of 5436 patients undergoing primary elective CABG surgery in order to determine the incidence of death in the first three post-operative days. A subset of 2666 patients was divided into two groups; those receiving preoperative statins and those not. A significant improvement in all cause mortality and cardiac mortality (all deaths in the early period were cardiac in nature) was demonstrated in the statin group in the first three post-operative days from 1.4% to 0.3% (p<0.01) however over the total hospital stay, no difference in mortality was seen. Again,

although a difference in early mortality was seen, there was no difference in peri-operative myocardial enzyme release. Interestingly, a multivariate analysis controlling for postoperative discontinuation of medications found that discontinuation of statins was independently associated with an increased risk of late postoperative cardiac mortality compared with those continuing with statin therapy (1.91% v 0.45%, p<0.01). This may correlate to observations in the laboratory setting where withdrawal of statins results in a rapid reduction in NO due to previously inhibited Rho Kinase becoming translocated to the plasma membrane and activated[170].

Recently a randomised study of rosuvastatin versus placebo in a selected population of patients undergoing CABG was performed. In contrast to previous studies patients treated for 7 days prior to CABG surgery with rosuvastatin showed significantly less myocardial injury perioperatively[169]. However, in that particular study, patients already taking a statin were required to stop treatment 30 days prior to the study protocol and all patients were only treated for 7 days pre-operatively and for an unstated post-operative period; this therefore does not reflect common clinical practice.

The mechanism of protection which statins appear to be affording in this case is not clear. Whether statins are having a direct myocardial protective effect in this setting is difficult to conclude from one study. A recent study looking at patients undergoing CABG on cardiopulmonary bypass randomised 30 patients to either simvastatin 40mg for 3 weeks prior to surgery or placebo and examined cytokine release as a measure of inflammation and the rate of neutrophil apoptosis. They found a reduction in IL-6 and IL-8 and an increased rate of neutrophil apoptosis. Their hypothesis being that the inflammatory effect of the CPB increases neutrophil functional activity, preventing their normal rate of turnover and causing a prolonged inflammatory effect. Statins acting to reduce this prolonged neutrophil activity

and hence reducing the inflammatory state, may explain the differences in mortality seen in those taking statins or not[166].

However, as discussed earlier it has been shown in animal based ischaemia/reperfusion injury studies that the direct myocardial protective effect of statins wanes over time[78] and this may explain the lack of difference in cardiac enzyme release. Crucially, in these animal studies it was possible to recapture the protective effect and this is an avenue which I have investigated in the setting of CABG surgery and the findings will be discussed later.

One study has been undertaken in cardiac valve surgery; a retrospective analysis of 447 patients undergoing a variety of cardiac valve operations compared 30 day outcomes of those taking or not taking a statin medication prior to the operation. Although the statin group had more risk factors for peri-operative complications, they had better outcomes, with the unadjusted odds ratio for the composite outcome of death, stroke and renal failure being 1.9 with a 95% CI of 0.95 to 3.76 (p=0.068). The authors acknowledge that this retrospective analysis requires validation by a randomised study but if confirmed this would extend the domain of the potential protection provided by statins[167].

A recent meta-analysis of preoperative statin use in cardiac surgery comparing data from 30,000 patients has shown significant benefits from preoperative statin use in early post-operative all-cause mortality, atrial fibrillation and stroke but conferred no benefit on postoperative myocardial infarction or renal failure[168]. There was a 1.5% absolute reduction in early postoperative death; however 95% of this effect was contributed to by the 16 observational studies compared against only 3 prospective studies. The overall mortality reported in the control groups was 3.7%, twice the current mortality in the United Kingdom (1.8%)[160] and so a further mortality reduction of 1.5% may be difficult to realise; although of course statin use may already be playing a role in UK's lower mortality figure.

At the present time, unless absolutely contraindicated, patients should be taking a statin medication prior to and following CABG and probably valve surgery and it should be continued during the operative period as much as is possible.

Statin therapy and non-cardiac surgery

Cardiac complications during and following non-cardiac surgery are common. In a group of 6237 unselected patients over 40 years old, undergoing a variety of major non-cardiac types of surgery, the average rate of myocardial infarction or cardiac death was 2.5% (range 2-3.7) and in patients with known vascular disease this increased to 6.6% (range 2.2- 19)[171]. Even when patients are known to have cardiac disease it is difficult to predict who will have a problem. Patients may not be symptomatic under normal circumstances but intra-operative or postoperative factors which change the ratio of myocardial supply and demand such as hypotension, hypertension, anaemia, vasoactive medications and the general inflammatory state of surgery may reveal previous quiescent coronary disease. Of postoperative deaths reported to the National Confidential Enquiry into Peri-operative Deaths (NCEPOD) in the United Kingdom that underwent a post-mortem examination in 1999, 36% died from cardiovascular causes[172]. There is once again an obvious need for interventions to reduce cardiac events and statins have been implicated as having a role to play (table 6).

Table 6: Statin therapy and non-cardiac surgery

Study	Study design	Number and type of patients	Intervention	Follow up	Outcomes
Poldermans, 2003[173]	Retrospective case controlled	2816 patients undergoing major vascular surgery.	160 cases matched with 320 controls Analysis of Statin use.	30 day mortality	Significantly ↓mortality rate in Statin uses against controls (OR 0.22, 95 CI 0.1 to 0.47)
Kertai, 2004[174]	Retrospective cohort	570 patients undergoing elective AAA repair	Analysis of pre-op Statin and β-blocker use or not.	In hospital and 30 day mortality and MI	Statin use independently associated with ↓mortality or post-op MI (OR 0.24, 95% CI 0.1 to 0.7, p=0.01)
Lindenauer, 2004[175]	Retrospective cohort	780,591 patients undergoing major non-cardiac surgery.	70,159 Statin users with matched propensity analysis	In hospital mortality	Statin use assoc. with ↓crude mortality from 3.05 to 2.13% (p=0.001).
Durazzo, 2004[176]	Prospective, randomised, placebo controlled, double blind	100 patients undergoing major vascular surgery.	20mg atorvastatin for a mean of 30 days pre-op or placebo.	6 months	↓ adverse cardiac events from 26 to 8% (p=0.031).
O'Neil Callahan, 2005[177]	Retrospective cohort	1163 patients who had undergone major vascular surgery	Pre-operative Statin or not	In hospital	↓peri-op cardiac events from 16.5 to 9.9% (p=0.001). Mainly ↓myocardial ischaemia.
Feringa, 2007[178]	Prospective non-randomised.	359 patients undergoing elective major vascular surgery.	Pre-operative Statin therapy or not. Variety of types and doses	30 days and longer (mean 2.3 years)	Higher Statin doses significantly ↓post-op Troponin T release. Statins significantly ↓ 30 day and late post-operative cardiac events.
Schouten, 2009[179]	Prospective, randomised, placebo controlled, double blind	497 patients scheduled to undergo vascular surgery	80mg fluvastatin extended release or placebo started pre-operatively	30 days	↓ myocardial ischaemia from 18.9% to 10.9% (OR 0.53; 95%CI 0.32-0.88). ↓ cardiovascular death or non-fatal MI from 10.1% to 4.8% (OR 0.48; 95% CI 0.24-0.95)
Dunkelgrun, 2009[180]	Prospective, randomised, open label	1066 patients scheduled to undergo non-cardiovascular surgery	Patients allocated to bisoprolol, 80mg fluvastatin, combination therapy or	30 days	Bisoprolol ↓ cardiac death or MI (2.1% vs. 6.0% events; 95%CI: 0.17–0.67; *P*=0.002). Fluvastatin non-significantly ↓ cardiac death or MI (3.2% vs.

			neither (control)		4.9% events;95%CI: 0.35–1.10; P= 0.17)

An initial case control study suggested a large reduction in peri-operative cardiac complications in patients taking statins prior to non-cardiac vascular surgery with an adjusted odds ratio of 0.22 (95% confidence interval 0.1-0.47) between statin users and non-users[173]. Following this, the same group went on to show that statin use prior to elective aortic aneurysm repair, even after adjusting for covariates, was associated with a reduction in the combined end-point of peri-operative mortality and MI at 30 days follow-up (OR 0.31, 95% CI 0.13 to 0.74, p=0.01)[174]. A large retrospective cohort study of 780,591 patients undergoing non-cardiac surgery showed a reduction in crude mortality from 3.05% to 2.13% (p<0.01) in those taking regular statin medication and this difference persisted in the propensity matched cohort as well. This study was limited in drawing conclusions because they defined a patient to have received regular statin treatment if they received it on post-operative day 1 or 2 and so were not able to comment on duration of preoperative use definitively[175]. In the retrospective StaRRS study of 1,163 patients, regular statin use prior to non-cardiac vascular surgery reduced peri-operative cardiac complications from 16.5% to 9.9% (p=0.001) but the reduction was driven by a reduced incidence of myocardial ischaemia and congestive heart failure rather than total mortality[177].

Poldermans' group went on to demonstrate in a non-randomised prospective observational study of 359 patients that the intensity of statin therapy is also important when considering reductions in cardiac events during major non-cardiac vascular surgery. In patients undergoing elective abdominal aneurysm repair, peripheral arterial bypass surgery and carotid artery surgery, myocardial injury was measured by Troponin-T release and ischaemic episodes were recorded using continuous 12 lead ECG recording. Cardiac events were

recorded at 30 days and later (mean 2.3 years). Both, higher statin doses (regardless of LDL cholesterol concentration) and lower serum LDL cholesterol concentrations were associated with reduced myocardial ischaemia. In fact for each reduction in LDL cholesterol of 10mg/dl there was a 13% reduction in ischaemic events. They also reported a significant reduction in the rate of cardiac events (albeit small numbers) at 30 days with an odds ratio of 0.32 (95% CI 0.1-0.96) and in late cardiac events with a hazard ratio of 0.41 (95% CI 0.21- 0.75)[178].

A randomised double blind study by Durrazo et al.[176] allocated 100 patients about to undergo vascular surgery to 20mg of atorvastatin or placebo at least 30 days prior to their operation. The primary endpoint was of composite cardiac death, non-fatal myocardial infarction, unstable angina and stroke. Atorvastatin significantly reduced cardiac events over 6 months post-operatively from 26% to 8% (p=0.031). Although very promising, the total number of events was small (17) and as such further validation was needed.

DECREASE III was designed to fulfil this role. 497 statin-naive patients scheduled to undergo vascular surgery were randomised to placebo or 80mg fluvastatin extended release. At 30 days post-operatively myocardial ischaemia was detected in 74 patients and was reduced from 18.9% in the placebo arm to 10.9% in the fluvastatin group (OR 0.53; 95% CI 0.32-0.88) with a 52% relative risk reduction in the combined end-point of cardiovascular death or non-fatal MI (OR 0.48; 95% CI 0.24-0.95)[179]. This study suggests that there is a large benefit to be gained from fluvastatin XL in patients undergoing non-cardiac vascular surgery. This is unlikely to be an effect confined to fluvastatin and the majority of patients undergoing vascular surgery are likely to benefit from a statin in terms of their systemic atherosclerotic disease and placebo controlled trials may no longer be justifiable. Creative study design may certainly allow us to clarify the optimum timing and dosing of preoperative

statin therapy and whether or not the use of statins acutely prior to the operation could bring further benefit would be a very interesting basis for study.

Although major society guidelines are yet to be updated some general recommendations regarding statin use prior to non-cardiac surgery can be made. All patients prescribed statins for established primary or secondary prevention prior to any type of surgery should continue them perioperatively and these should be stopped for the shortest possible time peri-operatively. All patients with known atheromatous vascular disease undergoing vascular surgery should, if not already on a statin, be seriously considered for one and this should be commenced as early as possible prior to surgery and again should be continued peri and post operatively. At present we cannot advocate a statin prior to all surgical types however because the DECREASE IV study, designed to evaluate bisoprolol and fluvastatin in patients at intermediate risk of post-operative cardiovascular events, demonstrated a benefit of bisoprolol but only a non-significant trend to benefit with fluvastatin. It is likely that in this lower risk group, there is less to gain from statin medications in terms of atherosclerotic plaque stabilisation etc.[180][181] .

Conclusion

Statins are established as playing a major role in cardiovascular disease in almost all categories of patient risk. The 'pleiotropic' effects of statins continue to reveal further potential benefits in a diverse range of interventional settings. The benefit of using statins acutely prior to scenarios where myocardial injury may be expected and in emergent situations is slowly being realised. The potential benefits of limiting myocardial injury in these settings are evident and further work is needed to clarify the optimum statin regime in different settings and the mechanisms behind the cardioprotective actions. Whether or not further cardioprotective effects can be gained by additional acute doses of statins has exciting

potential and merits further study. Specifically, whether high-dose atorvastatin is beneficial in the setting of cardiac surgery has not been established (see chapter 3).

Erythropoietin as a myocardial conditioning mimetic

Erythropoietin (Epo) is a haematopoietic cytokine which acts via membrane bound receptors on erythroid progenitor cells to regulate red cell mass[182]. Recombinant versions (rhEpo) are well established in humans as a treatment for anaemia but fairly recently Epo has also been demonstrated to have cardioprotective potential. The different types of rhEpo are the α and β forms and a long acting analogue, darbepoietin α (DA). The different types have almost identical clinical haematopoietic effects although DA acts over a longer time period. The α and β forms have been reported as having some variation in biochemical response and that there may be some inter-batch variation in glycosylation within the same type. This does not appear to affect the haematopoietic characteristics but it is not known how interaction with the Epo receptor complex may vary to produce any cardioprotective effects[183]. Epo is reported as being able to reduce inflammation, inhibit myocyte apoptosis and stimulate vasculogenesis in order to limit infarct size and reduce detrimental myocardial remodelling in laboratory models of ischaemia and reperfusion injury. It appears that activation of the RISK pathway (figure 3), as described above, is again crucial to these beneficial effects. In the following section, the laboratory and clinical evidence for cardioprotection by Epo shall be reviewed with particular emphasis on its role in acute reperfusion injury.

The emerging cardioprotective effect of Epo

Beyond its role in haematopoeisis, Epo and its receptor have been demonstrated in a number of other cell types including all types of muscle cell, insulin producing cells, endothelial cells and neurons[184]. The identification of these alternative sites sparked renewed investigation into the abilities of Epo and particularly whether it could afford cellular protection in the nervous and cardiovascular systems. Epo was found to protect neurons and the brain against

ischaemic injury in 1998[185] but it was later in 2003 that a number of different groups demonstrated that Epo had cardioprotective effects (see below).

The time point that Epo is administered relative to the experimental I/R injury allows elucidation of the potential mechanism of protection but also, this time point can be correlated to the clinical setting where the cardioprotective effect may eventually be harnessed; for example, prior to an elective procedure where myocardial injury is anticipated, such as elective CABG surgery, or after the onset of ischaemia in an unexpected acute myocardial infarction. In the section below the evidence for cardioprotection at the different experimental time points is reviewed (see Table 7 for a summary of studies).

Epo administered 24 hours prior to ischaemia

Initially, investigators established the presence of the Epo receptor (EpoR) in isolated neonatal rat ventricular myocytes and then, when co-incubated with Epo, apoptosis induced by hypoxia was found to be significantly reduced[186]. Epo treatment 24 hours previously was found to significantly reduce apoptosis by 50% in isolated neonatal rat cardiomyocytes subjected to either oxidative stress or when subjected to 12 hours anoxia from 56.8% ±6.7% to 37.7% ±3.9% (p<0.01)[187]. Similarly, a study in isolated adult rat cardiomyocytes showed that Epo reduced apoptosis by approximately 50%[188]. Cai et al.[189] first demonstrated in 2003 that rats treated with 5000 U/kg of Epo 24 hours prior to I/R injury on an isolated Langendorrf rig showed improved functional recovery and a reduction in myocyte apoptosis. Yellon's group showed in an isolated rat heart model that hearts treated by Epo, '3 times a week' for 3 weeks were protected but not when treated only once, a week before I/R, although the same dose (5000 U/kg) administered just once, 24 hours prior to I/R was protective[190]. The infarct limiting effect of Epo administered 24 hours prior to I/R has also been demonstrated in a rat in-vivo recovery model[191][192] but not by all[193]. Burger et al. have

used a mouse in vitro[194] and in vivo model[195] in order to demonstrate that Epo significantly reduces apoptosis and therefore subsequent infarct size at this time point, and that p-38 MAPK mediated heme oxygenase-1 (HO-1) expression is important in this effect[195].

Epo administered at the onset or just after ischaemia

Initial studies with Epo administered at this time point looked at the effects of permanent coronary artery occlusion without reperfusion. In the rat[186 196 197] and the rabbit[187] Epo was shown to reduce apoptosis and improve functional recovery, although repeated doses achieved no further benefit than just the first dose[197]. Although benefit in a permanent ligation model was an exciting development, models exploring the effects of Epo with reperfusion more closely mirror the human clinical setting. Epo given at or just after the onset of ischaemia in infarct models with reperfusion has shown reduced apoptosis and infarct size in rat in-vitro[198-202] and in-vivo models[188 203-207]. One large mammal study has been conducted by Kristensen et al.[208] using a catheter based porcine coronary occlusion model where 35,000U rhEpo was administered intravenously either 90mins or 24 hours (intramuscularly) and 90 mins before (intracardiac) 45 mins I and 150 mins R. Although in the latter group there was an improvement in LV functional parameters (dp/dt_{max}), there was no difference shown in infarct size between the control and treated groups, which appears to contradict the findings in the smaller animals.

Epo administered at reperfusion

Cardioprotective effects of Epo at reperfusion would have the potential to be translated to a number of elective and emergency settings of myocardial reperfusion injury in humans. The first study at this time point in a rat in-vivo model gave rhEpo (5000 U/kg) following 30 mins I and just before 4 hrs reperfusion and then daily doses for 7 days. Interestingly at the 4 hour

time point no difference in infarct size was shown, although at 7 days the treated group had improved LV function as demonstrated by a reduced LVEDP, suggesting that beneficial remodelling had taken place despite no difference in initial infarct size. However following this study, treatment with Epo at reperfusion has shown significant reductions in infarct size, apoptosis and improvements in haemodynamic measurements in rat in-vitro models[202 209 210], in-vivo models[205-207 209], rabbit in-vitro[211] and in-vivo[204] models. Of the larger mammals studied canine, porcine and ovine models have been used. In the canine model[212] 100 U/kg and 1000 U/kg doses of rhEpo were given just before reperfusion and both showed a significant reduction in infarct size, incidence of lethal arrhythmias and in myocyte apoptosis (although a greater effect was seen with the higher dose). However, in the porcine and ovine models such impressive results were not seen. Toma et al.[213] used a porcine catheter based model to occlude the LCx coronary artery for 60mins and administered darbepoietin (30µg/kg) intravenously at reperfusion. Following 2 weeks of reperfusion there was no difference in infarct size seen using histology but they did demonstrate moderate beneficial remodelling in the peri-infarct zone, with decreased fibrosis, increased capillary density and increased regional wall motion. Olea et al.[214] used an ovine catheter based coronary occlusion model and 3000 U/kg of rhEpo was administered at reperfusion and 24 and 48 hrs later. No difference in infarct size was seen as assessed by morphometry or hydroxyproline methods and using echocardiography and LV catheterisation, no difference in EF was found. In fact in the treated group there was an increased LVESV and LVEDP, suggesting LV dilatation and adverse remodelling at 10 weeks post infarction. A further step towards the human setting has been taken by Yellon's group who have investigated rhEpo given at reoxygenation in a human atrial trabeculae model of I/R. The atrial appendage from patients undergoing cardiac surgery on cardiopulmonary bypass was harvested and atrial trabeculae isolated and suspended in a superfused organ bath and the developed contractile force measured. In this

way the trabeculae were subjected to hypoxia and reoxygenation with rhEpo (50ng/ml) perfused at reoxygenation and it was reported that the treated group had a significantly improved recovery of contractile force than controls and this coincided with decreased caspase 3 (as a measure of apoptosis) in the treated group which was mediated via PI3k and ERK 1/2[215].

Delayed Epo administration

A number of investigators have explored the protective effects of rhEpo given after the initial reperfusion with further doses at delayed time points, or in permanent ligation models where there may be no reperfusion and Epo is administered quite sometime after ischaemia. Van der Meer et al.[216] found that although darbepoietin (40μ/kg) administered at the onset of permanent coronary artery ligation reduced infarct size at 9 weeks, this was not the case with delayed administration 3 weeks following the index ischaemia. However at both time points of dosing, significant improvements were seen in haemodynamic measures of LV function and increased capillary density noted. Westenbrink et al.[217] confirmed these findings, where in a similar model, dosing at 3 weeks post coronary artery ligation did not reduce infarct size but significantly improved the haemodynamic measurements. However, at time points closer to reperfusion than 3 weeks, substantial benefits in infarct size have been realised as well. In a rat permanent ligation model a significant reduction in infarct size at 4 weeks was demonstrated with rhEpo up to 12 hours after ischaemia as well as improved LVEF. Epo given at 24 hours, did not reduce infarct size but did reduce apoptosis[218]. However in a canine permanent ligation model a reduction in infarct size was only seen when rhEpo was given at the onset of I, although dosing at 6 hours post I did produce significant haemodynamic benefits, increased circulating endothelial progenitor cells and improved capillary density; treatment at 7 days post ligation produced no benefit at the 4 week assessment[219]. Although in

reperfused rat models a reduction in infarct size and beneficial remodelling has been reported with DA administered 24 hrs[220] and 7 days[221] after infarction.

Table 7: Overview of all animal or human tissue studies investigating the effect of Epo on infarct size or apoptosis following ischaemia-reperfusion or permanent coronary artery occlusion.

First author	Date	Model	Treatment regime	Key results	Mechanistic insight
Administration of Epo ≥ 24 hours before ischaemia[a]					
Cai [189]	2003	Rat *in vitro*, 30 min global I[b] / 45 min R[c]	rhEpo (5000 U/kg) ip 24 h before I	↑ LVDP[d], ↓ apoptosis	↓caspase 3 activation
Bullard[190]	2005	Rat *in vitro*, 35 min I / 120 min R	rhEpo (5000 U/kg) sc three times a week for three weeks before I	↓ IS[e]	↓ IS dependent on NOS, but not PI3K
Hale[193]	2005	Rat *in vivo,* permanent ligation, 6 weeks	rhEpo (5000 U/kg) sc 5 days and just before I and daily for 5 days after I	= IS, ↑ EF[h]	
Liu[191]	2006	Rat *in vivo*, 30 min I / 3 h R	rhEpo (5000 U/kg) ip 24 h before I	↓ IS	↓ myocardial proinflammatory cytokines, ↓ neutrophils
Liu[192]	2006	Rat *in vivo*, 30 min I / 3 h R	rhEpo (5000 U/kg) ip 24 h before I	↓ IS	↑ COX-2 mRNA and PGE_2 and 6-keto-$PGF_{1\alpha}$ ↓ IS dependent on COX-2
Burger [194]	2006	Mouse *in vivo*, 45 min I / 3 h R	rhEpo (2500 U/kg) iv 24 h and 30 min before I	↓ IS, ↓ apoptosis	↑ myocardial eNOS expression. No ↓ IS in eNOS -/- mice
Burger [195]	2009	Mouse *in vitro* 90min I/ 90min R; *in vivo*, 45 min I/ 3h R *in vivo* 45 min I/ 6h	rhEpo (20 U/ml) for 24hours prior to I/R rhEpo (2500 U/Kg) iv 24h and 30 min before I. rhEpo (2500 U/Kg) iv 5 mins after I	↑HO-1, ↑CO, ↓ apoptosis. ↑HO-1 in WT mice and eNOS^{-/-} mice. =apoptosis and ↑IS in HO-1^{-/-} mice. ↓IS in WT	Anti-apoptotic effect mediated via HO-1 and CO. HO-1 expression mediated via p-38 MAPK and Akt but not Erk. ↑HO-1 independent of eNOS.

First author	Date	Model	Treatment regime	Key results	Mechanistic insight
		R			

Administration of Epo immediately before or after start of ischaemia					
Tramontano [186]	2003	Rat, *in vivo*, 60 min of I, no R	rhEpo (5000 U/kg) ip at time of I	↓ apoptosis	Akt dependent
Moon [196]	2003	Rat, *permanent ligation* 8 weeks	rhEpo (3000 U/kg) ip immediately after start of I	↓ IS, ↓ apoptosis (at 24 h), ↓ [f]LVEDD, ↓ [g]LVESD, ↓ thinning LV wall, ↑ EF, = mortality	No change in haematocrit
Parsa [187]	2003	Rabbit, *in vivo*, permanent *ligation* 4 days	rhEpo (5000 U/kg) iv at time of I	↑ peak LVDP, ↑ dP/dt$_{max}$ in response to isoproterenol, ↓ apoptosis (at 6 h)	↑ phosphorylation of Akt and ERK No change in haematocrit
Cai[198]	2004	Rat *in vitro*, 30 min global I / 45 min R	Perfusion with rhEpo (100 U/kg) for 15 min before I	↑ LVDP, ↓ [i]LVEDP, ↓ apoptosis	↑ phosphorylation of Akt. Effects mediated by PI3K
Van der Meer [199]	2004	Rat *in vitro*, 40 min global ischaemia / 2 h R	Perfusion with rhEpo (10 U/ml) starting before I until end of R.	↑ LVDP, ↓ apoptosis	↑ phosphorylation of ERK1/2, but not of STAT5
Kristensen [208]	2005	Pig *in vivo*, 45 min I / 150 min R	rhEpo (35000 U) iv 90 min before I or 24 h (im) and 90 min before I (ic)	= IS, ↑ dP/dt$_{max}$ (in latter group)	
Moon [197]	2006	Rat, *permanent ligation* 4 weeks	rhEpo (3000 U/kg) iv immediately after ligation as single dose, or daily for 6 days afterwards	↓ IS (only significant in single dose group), ↓ LVEDP, ↑ EF, = mortality	No benefit with repeated dosing.
Miki[200]	2006	Rat *in vitro*, 25 min global I / 2 h R	rhEpo (5 U/ml) for 15 min before I	↓ IS	↑ phosphorylation of JAK2 and Akt; ↓ IS dependent on PI3K, guanylyl cyclase, and mitoK$_{ATP}$

First author	Date	Model	Treatment regime	Key results	Mechanistic insight
Nishihara[203]	2006	Rat *in vivo*, 20 min I / 120 min R	rhEpo (5000 U/kg) iv 15 min before I	↓ IS	↑ phosphorylation of Akt, STAT 3, GSK-3β; ↓ IS dependent on PI3K and PKC (NS);
Miki[201]	2007	Rat *in vitro*, 25 min global I / 2 h R	rhEpo (5 U/ml) for 15 min before I	↓ IS	↑ phosphorylation of Akt and ERK1/2; ↓ IS not dependent on ERK1/2.
Ohori[222]	2008	Rat *in vitro*, 2, 6 or 24hrs of oxidative stress.	rhEpo (10 U/ml) 1hr prior to oxidative stress.	↓apoptosis ↑ Ser9 phosphorylation of GSK-3β in the mitochondria. ↓BAX translocation to mitochondria	EpoR and β subunit present in H9c2 cells. Protection mediated via PI3k/Akt to phosphorylate mito GSK-3β and prevent BAX translocation.
Administration of Epo at reperfusion					
Calvillo[188]	2003	Rat *in vivo*, 30 min I / 4 h R or 7 days R	rhEpo ip, 24 h and 0.5 h before I (2500 U/kg) or at R (5000 U/kg), and daily for 7 days	= IS (at 4 h), ↓ LVEDP (at 7 days)	
Parsa[187]	2003	Rabbit, *in vivo*, 30 min I / 3 days R	rhEpo (5000 U/kg) iv at time of R	↓ IS	Akt dependent
Parsa[204]	2004	Rabbit *in vivo*, 30 min I / 3 days R	rhEpo (1000 U/kg) iv 12 h before I or imme-diately before I or R	↓ IS (both groups), ↓ LVEDP, ↑ + dP/d$t_{max,}$ (only when given before I), ↓ apoptosis	↑ phosphorylation of JAK2, STAT3/5, ERK, and Akt
Lipsic[205]	2004	Rat *in vivo*, 45 min I / 24 h R	rhEpo (5000 U/kg) ip, 2 h before I, at time of I, or at R	↓ IS (all groups), ↓ apoptosis, ↓ LVEDP	↓ caspase 3
Bullard[209]	2005	Rat *in vitro*, 35 min I / 120 min R; *in vivo*, 40 min I / 24 h R	rhEpo (50 ng/ml) for 20 min 5 min before R (*in vitro*); rhEpo (5000 U/kg) ip at R (*in vivo*)	↓ IS	↑ phosphorylation eNOS, Akt (NS), and ERK1/2 (NS). ↓ IS dependent on PI3K and ERK 1/2

First author	Date	Model	Treatment regime	Key results	Mechanistic insight
Hirata[212]	2005	Dog *in vivo*, 90 min I / 6 h R	rhEpo (100 or 1000 U/kg) iv before R	↓ IS, ↓ incidence of ventricular fibrillation, ↓ apoptosis	↑ phosphorylation of Akt. ↓ IS dependent on PI3K
Hanlon[202]	2005	Rat *in vitro*, 20 min I / 40 min R (LVDP), 30 min I / 120 min (IS)	Perfusion with rhEpo (10 U/ml) for 10 min before I or at R	↓ IS, ↑ LVDP	↑ phosphorylation of Akt, ERK1/2, and translocation of PKC-ε; ↓ IS dependent on PKC (during I), and PI3K (during R)
Olea[214]	2006	Sheep *in vivo*, 90 min I / 10 weeks R	rhEpo (3000 U/kg) iv at time of R, and 24 and 48 h later.	= IS, = EF, ↑ LVESV, ↑ LVEDP	
Chan[210]	2007	Rat *in vitro*, 40 min global I / 120 min R.	Perfusion with rhEpo (5 U/ml) at 5 min before R until end of R.	↓ IS, ↑ LVDP	↑ phosphorylation of JAK2, ERK, ↓ expression of MMP-2 and 9. ↓ IS dependent on ERK.
Toma[213]	2007	Pig *in vivo*, 60 min I / 2 weeks R	jDA (30 μg/kg) iv at R	= IS, ↓ peri-infarct fibrosis, ↑ capillary density, ↑ wall motion	
Baker[206]	2007	Rat *in vivo*, 30 min I / 2 h R	DA (0.25-25 μg/kg) iv 15 min before I, 15 min before R, and 10 s after R (optimal dose 2.5 μg/kg)	↓ IS for all time points of administration	↑ phosphorylation of ERK1 and p38 MAPK, but not Akt ↓ IS (given before I) dependent on ERK1/2 and p38 MAPK, mito- and sarcK$_{ATP}$ channels, but not PI3K
Doue[207]	2008	Rat *in vivo*, 20 min I/ 90min R	rhEpo (200U/kg) iv at reperfusion. rhEpo (200U/kg) iv just before 20 mins I, 30mins R.	↓apoptosis ↓LVEDD and ↑FS at 2 and 4 weeks.	
Mudalagiri[215]	2008	Human atrial trabeculae	rhEpo (25, 50 and 150 ng/ml). 90 mins I, 120mins R. Optimal dose of 50 ng/ml at reoxygenation.	↑contractile force ↓caspase 3 activation	Dependent on PI3K and ERK 1/2.

First author	Date	Model	Treatment regime	Key results	Mechanistic insight
Kobayashi[211]	2008	Rabbit *in vitro*, 30min I/120 min R	Perfusion with rhEpo (1 U/ml) for 20mins until I rhEpo (10 U/ml) 25mins after I for 65mins.	↓IS, ↑p-Akt in mitochondrial fraction. =IS	Epo induces translocation of Akt to the mitochondria and protects when given pre-ischaemia Epo failed to protect at reperfusion
Delayed administration of Epo after reperfusion					
Van der Meer[216]	2005	Rat, *permanent ligation* 9 weeks	DA (40 µg/kg) ip once at time of I (early), or every 3 weeks, starting 3 weeks after I (late)	↓ IS (early), = mortality, ↑ LVDP (late), ↓ LVEDP (both), ↑ capillary density (both), ↑ dP/dt_{max} (late)	
Moon[218]	2005	Rat, *permanent ligation* 4 weeks	rhEpo (150 or 3000 U/kg) iv immediately after start of I or 4, 8, 12, and 24 h after onset of I	↓ IS, = mortality, ↓ LVEDV, ↓ LVESV, ↑ EF, ↓ apoptosis (at 24 h). 150 and 3000 U/kg equally protective.	3000 U/kg equally effective when given 4 ,8, or 12, but not 24 h after onset of I
Hirata[219]	2006	Dog, *permanent ligation* 4 weeks	rhEpo (1000 U/kg) iv at time of ligation (early) or after 6 h or 1 week (late)	↓ IS (only early EPO), ↑ LVEF (early and 6 h), ↓ LVEDP (early and 6 h), ↑ in circulating EPC's (early and 6 h), ↑ capillary density (early and 6 h)	=VEGF
Prunier[221]	2007	Rat *in vivo*, 40 min I / 8 weeks R	DA (1.5 µg/kg) ip once a week, starting 7 days after IR	↓ IS, = mortality, ↓ thinning of anterior wall, ↑ dP/dt_{max}, ↑ capillary density, ↑ circulating EPC's	No increase in circulating EPC's by 0.75 µg/kg DA and no protective effects by this dose
Gao[220]	2007	Rat *in vivo*, 30 min I / 72 h R	DA (30 µg/kg) at start of I, start of R, 1 or 24 h after R	↓ IS for all groups, ↑ +/- dP/dt_{max} on isoproterenol, ↓ apoptosis (at 48 h)	Significant for all time-points of administration
Westenbrink[217]	2007	Rat, *permanent ligation* 9 weeks	DA (40 µg/kg) ip every 3 weeks, starting 3 weeks after LAD ligation	= IS, ↑ EF, ↓ LVEDD, ↓ LVEDP, ↑ LVDP, ↑ dP/dt_{max}, ↑ capillary density, ↑ circulating EPC's.	Homing of EPC's to LV wall ↑ myocardial VEGF expression

First author	Date	Model	Treatment regime	Key results	Mechanistic insight
Kobayashi[223]	2008	Rabbit *in vivo*, 30 mins I, 2 days, 14 days and 2 mths R.	rhEpo (1,500 U/Kg) sc given at reperfusion and daily for 5 days. Epo DDS[k] applied to heart just after reperfusion	=IS between systemic Epo and control. =EF/FS =IS at day 2, ↓IS, ↑EF/FS at 14 days and 2mths	EpoDDS had no systemic effects on haematocrit. Day 2:↑EpoR, pAkt, pGSK-3β, pStat3, pErk, ProMMP-1, VEGF, Bcl-2, =Bcl-X$_L$. Day 14: ↑EpoR, pAkt, pErk,ProMMP-1, =VEGF, Bcl-2, Bcl-X$_L$, pStat3, pGSK-3β.
Prunier[224]	2009	Rat *in vivo*, 40mins I and 8 weeks R	DA 5000 U/kg ip at reperfusion DA 5000U/kg ip at reperfusion then 300U/kg ip once per week	↓IS ↓IS, =incidence of thrombus	No increased thrombogenicity of chronic Epo in this setting.

[a]When EPO was given before ischaemia as well as at reperfusion, the study is classified under "Administration of EPO at reperfusion"; when EPO was given before reperfusion as well as delayed after reperfusion, the study is classified under "Delayed administration of EPO after reperfusion". [b]ischaemia; [c]reperfusion; [d]LV developed pressure; [e]infarct size; [f]LV end diastolic dimension; [g]LV end systolic dimension; [h]ejection fraction; [i]LV end diastolic pressure; [j]darbepoetin, [k]drug delivery system

Summary

As demonstrated above, there is a wealth of evidence in small animal models (and one human tissue model[215]) of I/R that Epo can provide significant cardioprotection when administered at a number of time points either prior to ischaemia, after the onset of ischaemia, at reperfusion and at delayed time points often several days after reperfusion. This has produced a great expectation that this cardioprotection may be realised in the clinical setting. Tempering this hope however are the results from the experiments performed in larger mammals; these tend to be closed chest, catheter based, coronary artery occlusion techniques which appear to represent the clinical environment of either elective or emergency coronary artery intervention more closely than the external compression/ligation used in smaller animals.

Although the number of studies is small (3), a benefit in infarct size reduction has not yet been seen and there are mixed results regarding the later myocardial remodelling effects. The reason behind the failure to protect in larger mammals is not known. The incidence of arrhythmia during these experiments is quite high, but equal between the groups and thus is unlikely to introduce bias. The anti-arrhythmic medications used have been suggested as interfering with the protection, or perhaps a difference in the collateral circulation in these species is present, but no definite reason has been established and if these reasons are correct then it would make the translation of rhEpo as a cardioprotective agent to the human clinical setting rather challenging[208 214].

Despite mixed sentiments in terms of the potential translation to the clinical arena, all the studies described above have provided important insights in to the potential mechanisms of protection of Epo and these shall be described in the next section.

Potential mechanisms of cardioprotection by Epo

Epo has been reported to have a number of different beneficial actions which together contribute to its overall cardioprotection. These are its ability to reduce the inflammatory reaction, the formation of new myocardial blood supply, improved myocardial remodelling and a potent anti-apoptotic effect.

Anti-inflammatory effects

Myocardial ischaemia and infarction are potent inductors of inflammatory conditions within the affected area[225] particularly due to cellular necrosis, and as such, a reduction in inflammation has been suggested as a possible means of reducing injury and deleterious remodelling. However, it is not well established whether reduction in markers of

inflammation causes a reduction in cell death or is due to a reduction in cell death from an alternative protective mechanism. Epo does demonstrate an anti-inflammatory effect with a number of studies reporting cardioprotection by this mechanism[191][226]. This is unlikely to be the predominant cause of cardioprotection as studies performed in isolated cell lines and isolated heart models are removed from any immune interaction and yet cardioprotection is still demonstrated. It has been argued however that Epo may prevent the myocyte adopting 'an inflammatory phenotype' which can occur despite removal from the immune system and be cardioprotective, with this effect being mediated via PI3K[226][227].

Neovascularisation and the prevention of adverse myocardial remodelling

Following a myocardial infarction, neoangiogenesis is an integral part of the remodelling process but the capillary network is unable to support the greater demands of the myocardium resulting in progressive loss of tissue, infarct extension and fibrous replacement. The ability to directly induce new blood vessel formation in the infarct bed (vasculogenesis) and proliferation of existing vasculature (angiogenesis) has been shown to decrease myocyte apoptosis, increase viable myocardium and produce a sustained functional recovery[228]. In this regard, Epo has demonstrated that it can induce significant increase in vascular endothelial growth factor (VEGF)[217][223], mobilise and improve the homing of endothelial progenitor cells[217][219], reduce peri infarct fibrosis[213] and result in an increased capillary density seen at varying time points post infarction[216][217].

Anti-apoptotic actions

As previously described, the myocyte death caused from I/R injury is a combination of necrosis and apoptosis with a significant contribution coming from apoptosis[229] and with apoptosis continuing to occur late in to reperfusion (72hrs) whereas necrosis has peaked by

24 hours[230]. This would suggest that interventions, including Epo, directed at inhibiting apoptosis would be expected to have an effect some hours after I/R, which has indeed been demonstrated by the studies discussed above. Epo in its haemopoeitic role increases red cell mass by preventing apoptosis in erythroid progenitor cells[182], it also prevents neuronal apoptosis[231], and thus this action could be suggested to account for much of its cardioprotective effect as well.

Cell survival signalling and Epo

Epo is able to act directly at the level of the cardiomyocyte as demonstrated by a number of experiments in isolated cell lines[186-188 232]. The majority of its haematopoietic actions are mediated via the classical EpoR which has been demonstrated in the human heart[233], but the existence of a common β subunit heteroreceptor has also been demonstrated and is thought to mediate the direct cellular protective effects[234] (although protective effects not requiring the β subunit have been demonstrated with darbepoietin[235]) . This is evidenced by the fact that engineered analogues of Epo which do not activate the classical Epo R and demonstrate no haematopoietic effects are still able to activate tissue protection from I/R through their action at the β subunit[232 236 237] .

Following interaction with its membrane bound receptor, Epo activates a number of intracellular survival pathways.

Epo and the Reperfusion Injury Salvage Kinase (RISK) Pathway

As described previously the RISK pathway (figure 1) appears pivotal to cardioprotection induced particularly at reperfusion[46]. Epo activates this pathway with phosphorylation of MEK1/2- PI3k-Akt-Erk1/2 demonstrated in isolated cells[186 187], ex-vivo[189] and in-vivo[187 237] models although protection is thought to rely more heavily on Akt as targeted blockade

abrogates protection whereas blockade of Erk does not[195]. The end result is an inhibition of the opening of the mPTP via activation of eNOS, GC and PKC-ε and opening of mitochondrial K_{ATP} channels[200 202 203]. An increase in the threshold of mPTP by Epo has been shown to limit apoptosis[238].

The JAK-Stat pathway

Once JAK1/2 are activated[200 239], these in turn activate STAT-3[203] and STAT-5[204] (but not STAT-1α or STAT-5b[239]) which will eventually increase transcription of eNOS and COX-2, both of which have infarct limiting roles[192 194].

Alternative Akt mediated effects

Epo can induce translocation of Akt to the mitochondria and phosphorylate GSK-3β[211] (which is thought to occur at the serine 9 position[222]) and directly inhibit mPTP opening. pGSK -3β may also prevent cell death via inhibiting access of Bcl-2 associated X protein (BAX) to the mitochondria and inhibiting its pro-apoptotic effects[222].

The application of Epo as a human cardioprotective agent

Steps in this direction were stimulated following the demonstration that Epo may provide neuroprotection following acute stroke[240]. In the efficacy arm of this study 40 patients were randomised to rhEpo-β (33,000U) administered within 5 hours of symptom onset and for 2 days following this or placebo. Functional outcome was significantly improved at 1 month and there was a non significant trend to infarct size reduction as measured by MRI. As a translational step Yellon's group have used a unique human atrial trabeculae model to demonstrate that Epo is cardioprotective against I/R when infused at reperfusion[215]. Increasing evidence was added by Namiuchi et al.[241] who demonstrated that in 101 patients

with STEMI presenting for PPCI, those with higher endogenous serum Epo levels were likely to have smaller myocardial infarct sizes as measured by peak and total CK release, although the Epo level was not an independent predictor of infarct size. Serum Epo levels have also been examined in 67 patients undergoing cardiac surgery using cardiopulmonary bypass. Serum Epo levels were measured preoperatively and cardiac Troponin I measured over the 3 post-operative days was used as a correlate of myocardial injury but no relationship was found[242].

Epo as an adjunctive treatment to current management of acute coronary syndromes

In 2006 Lipsic et al.[243] published a small safety and efficacy study examining the potential cardioprotective effect of darbepoietin in STEMI. 22 patients were randomised to 300U of darbepoietin plus standard care or standard care alone prior to undergoing primary percutaneous coronary angioplasty. There was no difference in adverse events between the groups and no difference in EF at 4 months. There was a significant increase in endothelial progenitor cells in the treated group. Interestingly there was a non-significant trend to harm in the Epo group in the measured peak CK (1077 vs 487 U/L, p=0.22) and CK-MB (101 vs 47 U/L, p= 0.09).

In the setting of NSTEMI the cardioprotective effects of Epo have also been investigated. 51 patients were randomised to 40,000 U of rhEpo-α or placebo within 8 hours of a positive Troponin I result (>0.2 μg/l). No significant difference was shown in any cardiac enzyme and over the 18mths follow up there was no difference in major adverse cardiac events. The authors did note a significant increase in BP 2-4 hours following Epo administration but it was not reported whether this was transient or a longstanding phenomenon[244]. The timing of the Epo administration in this study is difficult to gauge. Patients must already have had significant myocardial injury in order to be included in the study and inclusion was timed

from the knowledge of the Troponin result rather than from symptom onset. Although Epo in experimental models can afford protection late in to reperfusion, protection was more reliable and larger with administration time points up to and including reperfusion (see table 7), suggesting that future studies would be advised to study Epo before or at reperfusion.

Following this a paper presented at the 2009 ACC has investigated the role of Epo in patients with STEMI undergoing PPCI. The Regeneration of Vital Myocardium in ST-Segment Elevation Myocardial Infarction by Erythropoietin (REVIVAL-3) randomised 138 patients to 33,000units rhEpo-β just after the first balloon inflation and then at 24 and 48 hours following this or placebo. The study failed to show any difference in the primary endpoint which was MRI assessed LVEF at 6 months or in the main secondary end point of infarct size. The authors did highlight a non-significant trend to harm in the Epo group in the MACE rate at 6 months although as yet there is no further explanation (Ott, 2009 Unpublished). Subsequently, four small studies have examined EPO as an adjunct to reperfusion therapy in STEMI. Binbrek et al.[245] administered 30,000units of rhEpo-β to 236 patients prior to thrombolysis for STEMI and were unable to show any difference in myocardial infarct size as measured by CK-MB release. Ozawa et al.[246] administered 12,000units of rhEpo-β to 41 patients within 24 hours of successful PPCI and demonstrated an improved LVEF within the EPO group over 6 months (from 51.0±19.6% to 58.5± 15.0%, P=0.0238), but not in the control group (from 47.2± 16.2% to 51.3±18.2%, P=0.2292) but this was not significant between EPO and placebo and there was no difference in infarct size (SPECT) or MACE. Suh et al.[247] administered 50U/kg of rh-Epo-α to 57 patients just prior to PPCI for LAD territory STEMI. The low Epo dose was chosen in an attempt to prevent side effects from Epo use. Suh et al. were unable to demonstrate any difference in enzymatic infarct size, CMR parameters or MACE rates over 6 months, potentially due to the low dose Epo used which is

substantially lower than that used in pre-clinical studies. Ferrario et al.[248] recruited 30 patients with STEMI undergoing PPCI and randomised to 33,000units of rhEpo-β just prior to reperfusion and at 24 and 48 hours or placebo. The Epo group had increased numbers of CD34+ endothelial progenitor cells and gene expression was shifted towards anti-apoptotic, anti-inflammatory and anti-atherosclerotic up regulation. There was reduced CK-MB (AUC) over 120 hours but no difference in infarct size by CMR acutely and at 6 months. There was a reduced LVESV in the Epo group and improved regional wall thickening in the infarct territory, which has been suggested as improved LV remodelling in this group.

In summary, a number of studies have examined Epo use in STEMI with a variety of protocols varying in Epo dose, timing of administration, patient type and end-points assessed. Results have not been consistent. The animal data would suggest that higher acute dosing prior to reperfusion is more likely to be beneficial and there is a need for studies with this in mind to further clarify the effects of Epo in STEMI.

Table 8: Overview of all clinical studies investigating the cardioprotective effect of EPO in patients with acute coronary syndromes or myocardial ischaemia-reperfusion injury

First author, year of publication	Design	Type of patients (n)	Intervention	Follow up	Outcome
Lipsic[243], 2006	Randomised, open-label	[a]STEMI, referred for PCI (n=20)	DA (300 μg) iv before PCI	4 mo	= peak [b]CK, = LVEF at 4 mo, ↑ circulating EPC's at 72 h.
Liem[244], 2007	Randomised, placebo-controlled	nonSTEMI (n=51)	rhEpo-α (40,000 U) iv within 8 h of presentation	18 mths	= peak CK/peak troponin I, = cumulative CK, = event rate at 18mths Note: increase in [c]SBP 2-4 h after administration

First author, year of publication	Design	Type of patients (n)	Intervention	Follow up	Outcome
Mocini[242], 2008	Randomised, open-label	CABG[d] and CVS[e] (n=67) CABG (n=40)	Measurement of Epo rhEpo-α (40,000 U)iv immediately pre-op	3 days	No correlation between serum Epo levels and post-op Trop-I or CK-MB. =Trop-I and CK-MB post-op.
Ott, 2009 (unpublished)	Randomised, double blind, placebo controlled	STEMI treated by PPCI (n=138)	rhEpo-β (33,000U) iv at first balloon inflation and 24 and 48 hours following.	6 months.	No difference in MRI measured EF or infarct size.
Binbrek, 2009[245]	Randomised, open label	STEMI treated with thrombolysis (n=236)	rhEpo-β (33,000U) iv just prior to thrombolysis.	30 days	No difference in enzymatic infarct size (CK-MB), echocardiographic measures or MACE.
Ferrario, 2009[248]	Randomised, placebo controlled, double blind.	STEMI treated with PPCI (n=30).	rhEpo-β (33,000U) prior to reperfusion, 24 and 48 hours.	12 months.	↑CD34+ cells at 72hrs, ↑NFkB, VEGFR-2, EPO-R and AKT gene genes from 24 to 72 h. ↓ TP53, CASP3, IL12a genes. ↓CK-MB (AUC). ↓LVESV. ↑infarct regional wall thickening.
Suh, 2010[247]	Randomised, open label, placebo controlled	STEMI (LAD only) treated with PPCI (n=57)	rhEpo-α (50U/kg) just prior to reperfusion	6 months	No difference in CK-MB, MRI parameters or MACE.
Ozawa, 2010[246]	Randomised, single blind, placebo controlled	STEMI treated with PPCI (n=41)	rhEpo-β (12,000U) within 24hours after reperfusion	6 months	No difference in CD34+ cells, infarct size (SPECT) or coronary artery luminal diameter. ↑EF in EPO group.

[a]ST elevation myocardial infarction, [b]creatine kinase, [c]systolic blood pressure, [d]coronary artery bypass surgery, [e]cardiac valve surgery

Epo and cardiac surgery

As previously discussed, cardiac surgery using cardiopulmonary bypass represents another clinical setting where the heart is injured through ischaemia and reperfusion. Epo was initially used in this setting for its haematopoietic effects, generally with relatively long term dosing regimes in order try and limit peri-operative blood transfusion[249]. Although markers of cardiac injury were not routinely measured, adverse events were recorded and no significant difference in any type of event was reported. There was an increase in post-operative death in the Epo treated group but on review this was felt not to be treatment related. Further mechanistic studies in humans post-cardiac surgery have not found an increase in clotting risk with repeated dosing and so has been reported as safe in this setting[250]. Recently Mocini et al. have investigated whether 40,000 U rhEpo or placebo administered in the immediate pre-operative period can protect against the I/R injury sustained. 40 patients undergoing elective CABG were randomised and Troponin I and CK-MB were measured over the 3 post-operative days as the primary outcome. No difference was found in peak cardiac enzyme levels (Trop-I 1.7 ± 1.8 vs 2.6 ± 3.4, $p>0.05$ and CK-MB 19.6 ± 13.2 vs 17.1 ± 12.6) although it was not stated exactly when the peak level occurred and the authors did not have enough data at day 3 in order to plot a summary time course measure such as the area under the curve[242].

Epo during resuscitation

Cardiac arrest and subsequent resuscitation can be seen as another situation where the heart (as well as the whole body) is subjected to first ischaemia and then reperfusion. An interesting experiment in a rat model has shown improved cardiac function in rats resuscitated from ventricular fibrillation with concomitant administration of 5000 U/kg of rhEpo[251]. Although this has been tested from a neurological stand point in humans with

benefit[252], the potential cardioprotective role has not been established and would be a further avenue of study.

Potential barriers to the translation of Epo as a cardioprotective agent in humans

The use of rhEpo to treat anaemia (mainly in the setting of renal failure) is well established but the extent to which Hb is normalised appears to correlate to increased mortality, particularly in those with IHD and CCF[253]. Meta-analysis of studies in this population has raised the prospect of increased risk of uncontrolled hypertension and thrombotic complications[254]. Therefore care must be taken prior to using Epo as a therapeutic agent in cardiovascular I/R injury because both hypertension and thrombosis would have the potential to cause particular harm in this group. There are however strategies which can mitigate this risk in order to allow the safer use of Epo.

As shown in Table 7 a significant number of animal experiments have demonstrated that cardioprotection can be achieved in most species with a single dose of Epo, which is unlikely to have significant effects on haematocrit or other long term actions. In dogs, cardioprotection was achieved with a dose as low as 100 U/kg[212] and as such, finding a dose response effect for cardioprotection in humans would assist in keeping the exposure to rhEpo as low as possible. One could envisage the use of 1-3 doses around the time of I/R injury for cardioprotective effects without extended dosing. Concomitant use of prophylactic heparin in a cohort of patients receiving Epo and undergoing cardiac surgery was safe and ensured no difference in complications between groups and no difference in platelet reactivity[250]. A recent study in rats treated with DA in a model of I/R and subsequently subjected to a thrombogenic stimulus found no increase in venous thrombosis[224].

Particularly of concern to interventional cardiologists is the interaction of Epo with the antiplatelet effects of aspirin and clopidogrel which are so crucial for maintaining coronary artery stent patency. Tang et al.[255] investigated the effects of differing rhEpo doses on the individual anti-platelet effects of aspirin and clopidogrel. With doses up to 200 U/kg no effect was found. With the 400 U/kg dose the post aspirin prolongation in bleeding time was significantly blunted although there was no effect on clopidogrel. Unfortunately the effect on the combination of aspirin and clopidogrel or in addition to other antiplatelet agents such as the glycoprotein IIb/IIIa inhibitors was not explored. Therefore although these results should be borne in mind when considering studies of rhEpo in cardiology patients taking aspirin and clopidogrel, it is likely that the combination of antiplatelet agents and anticoagulants used would diminish any adverse effects. This does however warrant further study.

Alternative possibilities to traditional rhEpo exist; carbamylated rhEpo does not interact with the classical EpoR and therefore does not have haematopoetic effects but is able to exert tissue protective effects via the common β subunit[256] and has been shown to retain cardioprotective benefits with no change in haematocrit[238 257].

Epo may also be able to exert beneficial actions if administered locally rather than systemically. A novel gelatin based drug delivery system applied to the epicardial surface of the infarcted myocardium which released rhEpo over time, was found to reduce infarct size and increase EF at 14 days in a rat model of I/R with no effects on haematocrit[223]. Although not tested in humans, this system could be envisaged to have a role in cardioprotection during cardiac surgery.

Conclusion

The cardioprotective benefits of Epo have been extensively demonstrated in experimental models of I/R injury. The benefits have yet to be realised in the clinical setting although a number of studies appear to have been conducted safely in different patient groups. There are a number of ongoing studies around the world continuing to address this issue and it will be interesting to see how these results compare.

Evaluation of cardioprotection in the clinical setting

In order to be able to measure cardioprotection one must first be able to detect cardiac ischaemia and injury. In the clinical setting myocardial injury is commonly assessed using biochemical tests, by assessing the electrical activity in the heart using an electrocardiogram (ECG) and by assessing LV or RV myocardial function using either non-invasive imaging (echocardiography or MRI) or with invasive angiography less commonly. Therefore the ability to limit the extent of abnormality in any measurement of cardiac injury can be used as a measure of cardioprotection.

Biochemical markers of cardiac injury

The measurement of cardiac injury in the clinical setting is routinely made using biochemical markers of myocyte injury. The most commonly used cardiac enzymes currently are Troponin-T/I and the myocardial band of creatine kinase (CK-MB). Elevation of these serum markers allows confirmation of the diagnosis of myocardial injury and enables judgements to be made as to optimal management. There is a proportionality to the cardiac enzyme rise in that the greater the rise the greater the myocardial damage[258] and different cardiac enzymes will be elevated at different time points following ischaemia (figure 4), however cardiac enzyme rise alone is not usually enough evidence to make a diagnosis as sometimes non-cardiac conditions may cause a rise.

Figure 4: Time course of the appearance of cardiac biomarkers in the blood after acute myocardial infarction (AMI). [CV: coefficient of variation]. (Adapted from[259]).
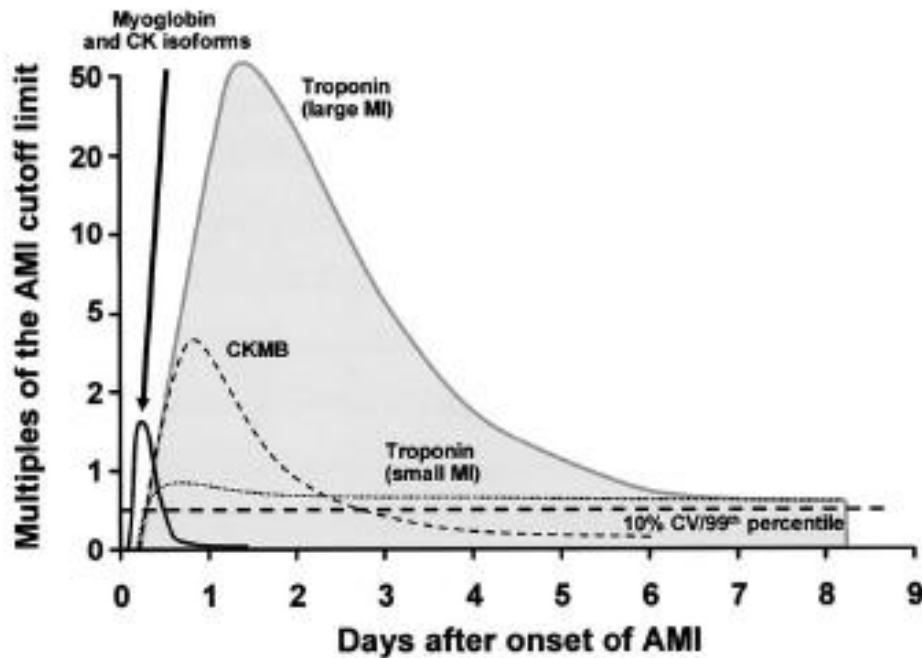
Electrocardiography

The electrocardiogram (ECG) is a common clinical tool. Abnormalities in the ECG trace are able to either hint at or give the diagnosis of myocardial ischaemia and the coronary artery or myocardial territory involved may be identified. Particularly in the case of ST segment shifts, where elevation suggests sudden acute ischaemia, the resolution of the ST segment shift can be used as a surrogate marker of cardioprotection. In that the protected myocardium subjected to ischaemia is less likely to cause such a rise in the ST segment[260].

Functional measures

Imaging techniques (either invasive or non-invasive) are able to measure either regional or global myocardial wall function. Long term outcomes become progressively worse with

reducing global LV/ RV functions and as such cardioprotective interventions which prevent myocyte death can be assessed on their ability to preserve LV or RV function and if successful may be expected to then improve longer term outcomes. Therefore LV function is commonly used as a surrogate measure of cardioprotection in studies.

Comparing cardioprotection between individuals

All surrogate markers of myocardial injury and cardioprotection need to be assessed for each individual in a clinical study and then compared across the study groups. No individual is identical and there is a need to normalise the relative success of cardioprotection in order to be able to interpret the findings. As such the measurement of the myocardial Area At Risk is important and shall be described below.

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Clinical myocardial area at risk assessment

</div>

What is the area at risk?

The myocardial area at risk (AAR) is the proportion of the myocardium jeopardised when the artery or arteries supplying this area are occluded[261]. If no reperfusion were to occur then the AAR would represent the maximal area of myocardial damage. When first described by Lowe et al. it was shown that for a given ischaemic time the eventual myocardial necrosis correlates to the AAR in animal models of I/R[261] and humans[262].

Why do we need to measure the AAR?

Following coronary artery occlusion, whether in experimental animal models or acute myocardial infarction in humans, myocardial injury starts. The progression of this injury is described as the 'wavefront phenomenon' as the cell death proceeds as a wave from

endocardial to epicardial sufaces[263]. Early on in the ischaemic time the lateral extents of myocardial necrosis are established on the endocardial border and do not extend. As the ischaemic time progresses the myocardial injury traverses towards the epicardium within the myocardial AAR[264]. However, if prior to maximal 'transmural' myocardial infarction reperfusion takes place (by whatever means) or other adjunctive therapies are initiated, then the wavefront progression of cell death may be halted and so 'salvaging' myocardium from the initial AAR[265 266]. The amount of myocardium salvaged is dependent on a number of factors but particularly the ischaemic time[267], presence of collateral circulation[268] and the adequacy of the restored perfusion[269].

When conducting studies on an intervention designed to limit myocardial injury it is possible to assess myocardial salvage in groups of patients via surrogate end points such as LV ejection fraction or cardiac enzyme release but by measuring the AAR in each individual and then comparing this to a more direct measure of eventual infarct size it is possible to gauge a much more accurate estimation of the effect of the trial intervention. In this way it is possible to control between individuals for variations in anatomy and the initial AAR, and assess confounding factors. Thus measurement of the eventual infarct size in relation to the AAR is a very attractive strategy for researchers and clinicians alike.

<u>Techniques to measure the myocardial area at risk in the clinical setting</u>

The measurement of the AAR has been continually evolving since its first description in 1978[261]. In the majority of animal experiments this is fairly straightforward as the perfused heart can be stained or marked in a number of ways and sectioned to allow accurate quantification of viable and infarcted areas. Besides post-mortem examination techniques it is not possible to measure the AAR in humans in such a direct way and so a variety of techniques are used as surrogates. These are broadly divided by the imaging modality they

rely on and have different strengths and weaknesses. The main modalities used include coronary and LV angiography, metabolic imaging methods, echocardiography, nuclear imaging and magnetic resonance imaging (MRI). In the following section the different methods will be briefly described with greater emphasis on two very utile angiographic methods and the emerging role of cardiac MRI in this field.

Angiographic methods for estimating the myocardial AAR

Coronary angiography

The use of coronary artery angiography and LV catheterisation is standard within cardiology. The need to be able to record the findings, standardise the report and explain the results to others led to the development of systems of scoring and describing abnormalities found such as that developed in 1977 at the Green Lane Hospital in Auckland[270]. Since that time a number of different coronary artery scoring systems have been described with their initial aim being to describe the total amount of myocardium at jeopardy from coronary artery disease and then to use this assessment to study and guide successful reperfusion strategies[271-273]. Despite slightly different methodologies between the systems the basic tenets remain the same. A score is attributed to a diseased artery in proportion to the size of the myocardial area at jeopardy that it supplies; in general a higher score correlates with, a higher burden of coronary atherosclerosis[272], a higher likelihood of ischaemic cardiomyopathy[271] and a worse prognosis[274]. By expressing the score for the area in jeopardy as a percentage of the score for the whole left ventricle a value for the proportion of the LV at risk is obtained, and therefore it is possible to compare groups of patients by controlling for the initial percentage of AAR. (See chapter 4 for the method and use of two angiographic risk scores).

LV angiography

Biplanar left ventriculography has been used for over 40 years as a method of measuring ventricular volumes[275]. By extending this method and assuming that any myocardium at risk during an acute MI does not contract normally then the LV myocardial area at risk can be estimated by assessing the proportion of abnormally contracting myocardium against that contracting normally. Whilst this technique has the advantages that it can be performed with the standard catheter laboratory equipment, is relatively quick and easy to do and the analysis can be done 'off line' retrospectively, it has been overtaken as a standard technique mainly because it has fairly high inter and intra observer variability[276] and performs poorly alongside newer techniques such as single photon emission computed tomography (SPECT) imaging[277] (see below).

Echocardiographic techniques

Echocardiography is an ultrasound technique which is well established for structural and functional imaging of the heart as it is safe, portable and can often be performed quickly. Its use in the clinical setting of acute MI to measure the myocardial AAR is less well established. Whilst it is possible to examine the regional wall motion of the LV, divide it in to segments and describe this to others, it does not really give a figure which is comparable between patients. The use of wall motion alone to evaluate AAR was found not to be particularly reliable as during acute MI, wall motion relies on other factors including volume and pressure loading conditions at the time[278]. However, the use of contrast agents to help examine perfusion of the myocardium has been much more promising. Initial animal experiments showed that in a canine model of coronary artery occlusion, contrast using hydrogen peroxide could allow echocardiography to delineate the non-perfused myocardium

and so the AAR at varying points through the heart could be assessed and that this correlated well with pathological comparison[279]. This technique has been extended to human use with the development of non-toxic contrast agents and has been well validated to a certain extent[280]. It has not however become a technique of choice because in the acute situation re-establishing coronary artery patency is the priority and there is little time to spend trying to get optimal echocardiographic views that can be interpreted 'offline' later and at present there is no echocardiographic technique to retrospectively establish the initial AAR. In this regard contrast echocardiography still has a role to play in evaluating myocardial microvascular perfusion but generally after the acute situation[280].

Nuclear Imaging Techniques

Nuclear imaging is commonly used to identify perfusion defects in the myocardium. Similar techniques can delineate the AAR. By injecting the radioactive isotope intravenously (commonly technetium or thallium or both) prior to reperfusion the area of myocardium affected will not take up tracer. Thus, when imaged with single photon emission computed tomography (SPECT) following reperfusion the resultant defect correlates well to the AAR[281]. A subsequent pre-discharge perfusion scan shows the size of the resultant infarct and therefore myocardial salvage can be calculated. Although this technique has been used with success it is logistically difficult as the radioactive isotope has a half life of 6 hours and there must always be a supply on hand in order to inject acutely prior to reperfusion in acute MI. The perfusion imaging must be done within 8 hours of the acute infarct and the isotope handled within radiation safety guidelines.

An alternative method which enables a longer time frame for imaging following MI is the use of ^{123}I-labeled 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP) which is an imaging radiopharmaceutical, which reflects fatty acid metabolism. BMIPP can be injected

even after reperfusion in acute MI, and it is possible to image the AAR, due to a lag in the recovery of fatty acid metabolism, up to 2 weeks following reperfusion[282]. This technique compares favourably with SPECT and does not have the disadvantages in terms of the short time frames needed but has yet to be adopted widely. One study has compared T2 MRI imaging (see later) to BMIPP for identification of acute myocardial injury and found greater accuracy with MRI[283] although no comparison has been made in imaging the AAR as yet. Glucose metabolism is also deranged following acute MI and so the positron emission tracer, ^{19}F-fluorodeoxyglucose has also been used to assess myocardial viability but has not been investigated in terms of AAR[284]. It has been suggested that further studies comparing metabolic imaging to MRI would be of interest[285].

Magnetic Resonance Imaging techniques

Cardiac MRI is rapidly evolving and finding increasing application, particularly with myocardial tissue characterisation. Two techniques are currently used to measure the myocardial AAR; the 'Endocardial Surface Area' measurement and T2 oedema imaging. These shall be described more fully in the next section.

Cardiac magnetic resonance (CMR) imaging

Theoretical Basis

At its most basic, magnetic resonance imaging involves placing the patient within a homogenous magnetic field (currently 1.5T) and applying radiofrequency pulses. A signal is generated from the release of energy of excited protons which is measurable and subsequently transformable in to a recognisable image. Different tissues have different rates at which the protons 'relax' or tend towards their original state, T1, T2 and T2*. By utilising

these different properties it is possible to use a variety of sequences which allow excellent tissue discrimination and tissue characterisation[286]. Application of MRI to the rapidly moving heart within the slowly moving chest was initially very challenging but advancements in technology and a number of techniques including ECG gating and repeated patient breath holding allows acquisition of optimal images.

Assessment of cardiac function

MRI is not constrained by anatomical 'imaging windows' such as those needed for echocardiography and so images can be obtained in any desired plane or orientation. This gives a great flexibility to tailor the examination to the patient. However there are number of images which are acquired almost as standard in order to describe and if necessary quantify LV or RV function.

Following initial 'scout' images a sequence of transverse slices through the chest are made to evaluate gross anatomy and cardiac position/axis. Cine images can then be taken in long and short axis to allow the acquisition of a traditional 'four chamber' view of the heart. Following this multiple cine image slices in short axis along the length of the ventricles from base (level of mitral and tricuspid valves) to apex allows a very accurate impression of LV/RV function to be made. A description of LV/RV function can not only be made in terms of broad overall ejection fraction but also in respect to individual regions, longitudinal function and the timing of contraction.

One of the great advantages of MRI is that beyond a narrative description of the ventricular function it is possible to measure with great accuracy and limited variability, end-diastolic, end-systolic and myocardial volume and from these calculate stroke volume, ejection fraction and myocardial mass[287]. In order to do this computer software is used to draw a 'region of

interest' (ROI), either manually or by predetermined signalling threshold methods, around the endocardial border at end-diastole and end-systole and a further ROI is drawn around the epicardial border (either at end-systole or diastole). Knowing the slice thickness allows one to calculate the stroke volume from the difference between total end-diastolic volume and total end-systolic volume and similarly the myocardial volume (and subsequently mass, using a conversion factor) can be calculated.

Thus accurate measurements can be made and importantly, because of the high reproducibility described above, changes over time can be reliably estimated. As there is no radiation involved, repeated scans do not pose a risk, which is particularly relevant in children with congenital heart disease who may require repeated scans over many years.

Tissue characterisation

By making use of the different behaviours of different tissues within the magnetic field judgements can be made about the composition and comparison to histological samples. By adding a contrast agent such as gadolinium which accumulates in the extra-cellular space and imaging at different time points post-administration, further information can be gleaned about myocardial perfusion, microvascular patency, extent of infarcted tissue and potential viability and the MRI data correlates well with known clinical parameters such as cardiac enzyme release[288] (see below for more detailed description of contrast enhanced imaging).

The use of cardiac MRI following acute myocardial infarction

In the very acute stages of myocardial function MRI currently has a limited role because of the pressing time issues and concerns over prompt reperfusion and patient safety. Although in cases of diagnostic uncertainty MRI has been suggested as a superior method to ECG, Troponin and TIMI risk score in discriminating cases of acute coronary syndrome[289].

However, in the days following acute MI valuable information can be gained which will guide clinical therapy and give researchers detailed information regarding cardioprotective techniques and whether these have provided a benefit or not.

LV remodelling

As described above accurate measurements of the LV/RV volumes and myocardial mass can be made. Following acute myocardial infarction, remodelling of the myocardium takes place to a greater or lesser extent in all people. Repeated scans enable these changes to be accurately mapped over time.

Perfusion

The rapid acquisition of images in the short axis plane, generally at 3 positions along the LV, whilst contrast is being injected, allows an assessment of myocardial perfusion. Normally this can be done at rest and stress (with exercise or pharmacological agent) to assess an inducible perfusion defect. In the short time following an acute MI it is inadvisable to subject the patient to a stress perfusion scan however rest images can easily be obtained. MRI can be used at an interval to assess the functional effect of any residual coronary stenosis noted on angiography.

Microvascular Obstruction

Imaging in the 'early' period following contrast administration enables visualisation of any microvascular obstruction (MVO) present from the acute myocardial infarction and is the optimal method of detecting intraventricular thrombus.

MVO is the result of injury sustained to the microvasculature during the period of ischaemia and reperfusion. MVO may occur despite adequate epicardial coronary artery

revascularisation and it is thought to result in the angiographic phenomenon of 'slow' or 'no reflow'[290]. MVO is caused by a number of factors including distal embolisation, microvascular inflammation and disruption and the activation of vasoactive mediators[291] and is thought to predominantly represent myocardial haemorrhage from red blood cell extravasation in to the extra-cellular space. The presence of MVO and myocardial haemorrhage has been shown to correlate to larger infarct size, increased adverse remodelling and reduced LV function over time following acute myocardial infarction[292] [293]. Optimal reperfusion should aim to restore micro as well as macrovascular flow and as such is a target for improvements in therapy. Measurement with MRI allows accurate assessment of strategies aimed at reducing MVO.

Infarct size

Imaging in the 'late' period (10-15 mins) following contrast administration allows images of the area of infarction to be obtained. Gadolinium is retained in the area of infarction for a longer period than either normal myocardium or blood. By adjusting the inversion time of the inversion recovery turbo fast low-angle shot (Turbo FLASH) MRI sequence the normal myocardium can be 'nulled' to allow maximum discrimination between infarct, blood pool and normal myocardium[294]. This technique has been shown to accurately correlate to pathological specimens and offers excellent specificity[294] [295]. It is as accurate as SPECT imaging but offers greater resolution and of course other information is obtained during the same scan[296]. Similarly to calculating other cardiac volumes, the ROI can be drawn around the infarct in each short axis slice and the total infarct volume/mass calculated. This can be expressed as a percentage either to the total LV volume/mass or to the AAR and can then be used as an end point in studies of cardioprotection. The extent of gadolinium enhancement correlates with functional recovery[297] and long term outcomes[298].

Measurement of the myocardial area at risk with cardiac MRI

There are 2 methods currently in use which attempt to quantify the myocardial area at risk. MRI is an ideal imaging modality with which to attempt to image the AAR because of its high spatial resolution and a large amount of different information can be obtained at one sitting. The AAR can be imaged following the acute MI so avoiding the need to complicate the acute situation and allowing the patient to be stabilised prior to scanning. The images can be analysed, 'offline' and blinded allowing detailed, unbiased evaluation- all attractive attributes for conducting studies of cardioprotection.

Endocardial surface area

This innovative technique described by Ortiz-Perez et al.[299] is relatively easy to perform and follows on from the 'wavefront' theory of infarct progression described by Reimer and Jennings[263] and discussed earlier. The acute infarct is imaged as described above. The lateral extents of the infarct and AAR are set early on in the ischaemic period and are delineated by the lateral extent of the gadolinium enhancement. The length of the infarct on the endocardial border is measured, multiplied by the slice thickness to give surface area and this is proportional to the AAR. This is expressed as a percentage of the total endocardial surface area of normal myocardium (calculated in the same way) to give a percentage of LV myocardium at risk (see figure 6, chapter 4). This has been validated against the two most common angiographic jeopardy scores and performs well (r=0.9 and 0.87, p<0.001)[299].

T2 imaging

Myocardial oedema has been recognised to develop following I/R injury for several decades and represents an increased water content both intra and extracellularly arising only in the

area of myocardium that was at jeopardy. Although it's exact cause remains uncertain it is thought to arise from an accumulation of osmotically active substances and a thermodynamic dysequalibrium contributing to the intercellular water influx[300]. Initial unsatisfactory sequences used to image myocardial oedema were improved greatly with the advent of T2 weighted short inversion-time, inversion recovery (STIR) imaging[301].

In dogs the area of oedema imaged using the T2 sequence correlates excellently with the AAR as measured by microsphere injection[302] and in pigs there is a similar excellent correlation with the fluroscein dye method (r=0.92)[303]. The extent of myocardial oedema in humans is greatest for up to a week following infarction and then gradually declines afterwards[304] allowing enough time following the acute event to enable retrospective determination of the AAR.

Although STIR imaging is able to provide fairly good tissue discrimination with reasonable signal-to-noise ratio there sometimes remains a problematic artefact with the blood pool remaining bright along the endocardial border. The development of a hybrid sequence called the Acquisition for Cardiac Unified T2 Edema Turbo Spin Echo Steady State Free Precession (ACUT$_2$E TSE-SSFP) sequence has managed to rectify this artefact in dogs at least, giving even more precise delineation of the area of myocardial oedema and hence the AAR[305]. This improved technique has yet to be validated in humans.

T2 STIR imaging has been compared against both BMIPP and SPECT imaging for the identification of acute myocardial injury and been found to be superior in identifying the culprit territory[283]. Very recently T2 STIR has been tested against the previous gold standard for AAR imaging, SPECT. 16 patients with acute MI received ^{99m}Tc tetrofusmin prior to PPCI and underwent SPECT imaging within 4 hours. T2 STIR MRI imaging was performed on day 1, day 7, 6 weeks and 6 months following reperfusion. Measurement of AAR by T2

STIR was found to be equivalent for imaging of the AAR for up to one week following acute MI[306]. This will undoubtedly be tested in a larger cohort of patients but it suggests that the AAR can now reliably be estimated, retrospectively, within a week of acute MI without the need to administer radiolabelled tracers and perform nuclear imaging in the acute period. It also prevents the need to perform a further predischarge rest nuclear perfusion scan to measure the eventual infarct size. This can now all be done with one scan at a convenient point within a week of infarction, meaning that MRI is likely to become the imaging modality of choice in order to assess the effect of cardioprotective interventions on myocardial salvage. It has also been shown in a canine model of ischaemia that myocardial oedema may develop during periods of ischaemia that may not cause cell death and subsequent infarction. The human analogy would be episodes of unstable angina. T2 imaging is able to detect the oedema and help delineate not only the myocardial territory of ischaemia but can quantify the area of myocardium at risk of further ischaemia in this area[307].

However, a few issues remain regarding MRI and T2 imaging for assessment of cardioprotection. Despite its relative acceptability, claustrophobia remains a significant obstacle and lying stationary and flat for scan times towards 45mins is difficult for some patients following acute MI. Another factor to be considered is the impact of the cardioprotective intervention being assessed on the extent of myocardial oedema itself. Of course, if the intervention reduces or increases the area of oedema relative to the eventual infarct size the myocardial salvage may be under or overestimated respectively and the impact of myocardial oedema on cell death still merits further study[308]. These observations notwithstanding, cardiac MRI is likely to continue to play a leading role in the continuing search for improved cardioprotective strategies.

Conclusion

We can see from the discussion above that both atorvastatin and erythropoietin have potential to limit myocardial injury in humans in the clinical setting. The assessment of cardioprotection in the heterogeneous clinical environment is not always easy with a number of different techniques needed. The next and subsequent chapters will discuss randomised trials of atorvastatin in cardiac surgery and erythropoietin in acute myocardial infarction performed in an attempt to reduce cardiac injury in these settings.

CHAPTER 2

Elucidating the Mechanistic Pathways of Ischaemic Preconditioning and Postconditioning in the Clinical Setting

The above title refers to a programme of studies investigating three pharmacological agents which target the Reperfusion Injury Salvage Kinase pathway. All three agents have been established in pre-clinical studies as able to limit lethal reperfusion injury and it is now important to investigate whether this is possible in the human clinical setting. Figure 1 below sets out the initial plan of investigating each agent in a variety of clinical scenarios.

Figure 1: Schematic to show overall initial plan of the study titled "elucidating the mechanistic pathways of ischaemic preconditioning and postconditioning in the clinical setting".
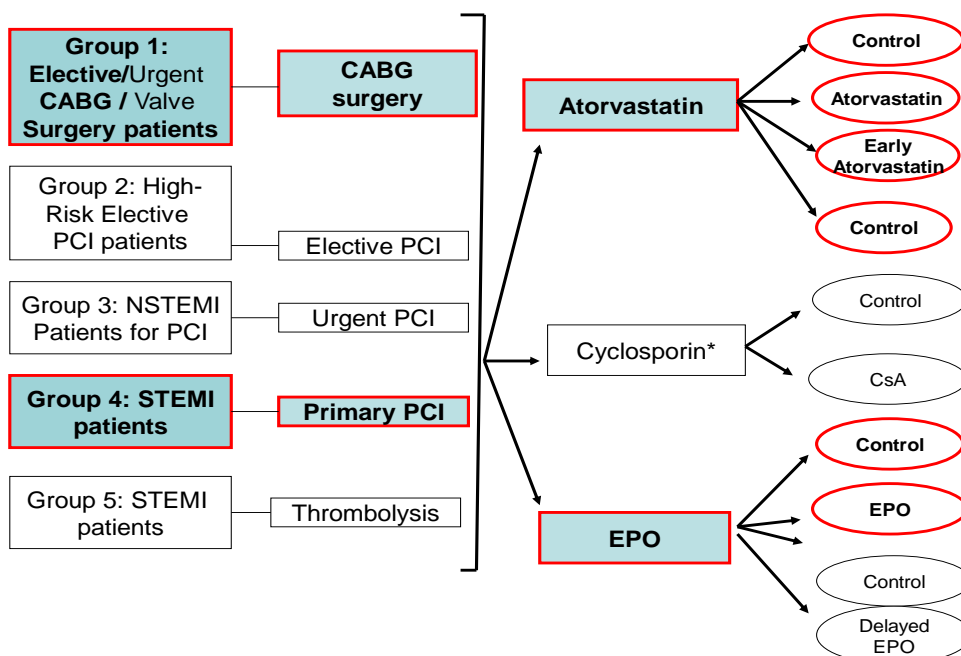
The highlighted sections in the figure above demonstrate the studies undertaken in this thesis; namely the use of atorvastatin in patients undergoing elective CABG and erythropoietin in patients with STEMI undergoing primary PCI. CABG- coronary artery bypass graft surgery, (N)STEMI- (non) ST elevation myocardial infarction, PCI-percutaneous coronary intervention, CsA- cycloporin, Epo- erythropoietin, TnT- Troponin T.

This thesis has taken a portion of the above study programme rather than the whole and therefore addresses the potential role of atorvastatin in elective coronary artery bypass graft surgery (group 1 patients) and erythropoietin in patients with acute ST elevation myocardial infarction treated by primary percutaneous coronary intervention (group 4). The other groups of patients and investigational agents have been addressed by other investigators.

As has been discussed, despite advances in revascularisation therapy in the elective and emergency setting, a considerable amount of myocardial injury may still occur and benefit can be gained if one can protect against this. Atorvastatin and erythropoietin are two agents which have been proven to act via a cell survival pathway (reperfusion injury salvage kinase pathway) to protect against IRI in preclinical animal studies. In order that this cardioprotective potential may be translated to the clinical setting, proof of concept studies are required to demonstrate safety and efficacy.

During coronary artery bypass surgery the heart is subjected to periods of ischemia followed by reperfusion causing myocardial injury which has been correlated to outcome[158][159]. In preclinical studies the protective effect of statin use has been demonstrated to wane chronically but could be recaptured with an acute high dose prior to ischaemia[78]. Whether this phenomenon can be reproduced to protect against myocardial injury during CABG surgery has not been tested.

Erythropoeitin has demonstrated significant cardioprotective effects in preclinical studies (see table 7, chapter 1) and acute high dose treatment has been safe in human pilot studies[240 243]. Whether high dose erythropoietin, as an adjunctive treatment to primary percutaneous coronary intervention in patients presenting with STEMI, is protective has not previously been established.

Hypotheses and Objectives

This thesis aims to investigate the use of atorvastatin and erythropoietin as a novel means of reducing myocardial injury in the clinical setting.

Hypothesis 1

Acute high dose atorvastatin on a background of chronic statin use administered prior to elective coronary artery bypass surgery will significantly reduce peri-operative myocardial injury.

Objectives

a. 160mg additional atorvastatin 2 hours prior to surgery and 24 hours following will limit myocardial damage as measured by cardiac Troponin T release over 72 hours following surgery.

b. 160mg additional atorvastatin 12 hours prior to surgery and 24 hours following will limit myocardial damage as measured by cardiac Troponin T release over 72 hours following surgery.

Hypothesis 2

High dose erythropoietin beta as an adjunct to primary percutaneous coronary intervention for ST elevation MI will reduce myocardial infarct size.

Objectives

a. Erythropoietin (50,000units rhEpo-β) administered prior to reperfusion and 24 hours after reperfusion to patients with ST elevation myocardial infarction (STEMI) as an adjunct to primary percutaneous coronary intervention (PPCI) will limit myocardial infarct size as measured by cardiac Troponin T release over 24 hours following reperfusion.

b. Erythropoietin as an adjunctive treatment in STEMI will provide beneficial effects as assessed by cardiac magnetic resonance imaging in the first week following reperfusion and at 4 months follow up:

   i. An increase in ejection fraction in the treated group.

   ii. Reduced adverse LV remodelling in the treated group, as evidenced by quantitative change in the LV end-diastolic and end-systolic volumes.

   iii. A reduction in myocardial infarction size as measured by quantification of the mass of delayed gadolinium contrast enhanced myocardium.

   iv. A reduction in the prevalence of microvascular obstruction in the treated group as assessed by early gadolinium contrast enhanced images.

The hypotheses presented above will be examined in clinical studies discussed in chapters 3 and 4.

<center>**CHAPTER 3**</center>

<center>**The Effect Of Acute High Dose Atorvastatin On Myocardial Injury During Coronary**</center>

<center>**Artery Bypass Graft Surgery**</center>

Introduction

Coronary artery disease remains the major cause of mortality and morbidity worldwide with more than 4 million deaths attributed to cardiovascular disease in the year 2000 in Europe alone[309]. Ischaemic heart disease is projected to be the leading cause of disability-adjusted life years by the year 2020 according to World Health Organisation estimates[1]. Although the mortality from routine adult elective coronary artery bypass graft (CABG) surgery is about 1.5% in the United Kingdom, the changing risk profile of patients being operated in terms of the aging population, increased prevalence of diabetes, and more complex operations, have resulted in mortality rates in the region of 10-20% in some high-risk groups[310]. Therefore, novel cardioprotective strategies are required to improve clinical outcomes in this patient group.

Mechanism of myocardial injury during CABG surgery

Myocardial injury sustained during cardiac surgery can be attributed to several different mechanisms, with acute myocardial ischaemia—reperfusion injury being the most important. Other causes include the inflammatory response to the extraneous substances in the cardiopulmonary bypass circuit, left ventricular over-distension, coronary atheroembolism, increased cardiac workload during the intraoperative period and direct myocardial injury due to retraction and handling of the heart[162]. Myocardial injury can be minimised by cardiac decompression, careful management of blood pressure, heart rate and systemic vascular

resistance intra-operatively and by exercising caution during manipulation of the heart and aorta.

Acute myocardial ischaemia—reperfusion injury during conventional on-pump CABG surgery results from the intermittent aortic cross-clamping required to undertake the attachment of each distal coronary anastomosis, resulting in cumulative episodes of global myocardial ischaemia. In patients undergoing a cardioplegic strategy, the cardioplegic solution is administered initially and repeated during each episode of aortic cross-clamping; or a continuous cardioplegia strategy may be used. In the technique of cross-clamp fibrillation an alternating current is applied to the myocardium to induce ventricular fibrillation. Ventricular fibrillation during perfusion results in an increase in the left ventricular end-diastolic pressure (LVEDP) causing subendocardial hypoperfusion[311], however this rise in LVEDP does not occur during ischaemia[312]. Therefore aortic cross-clamping and ventricular fibrillation appear to obviate the detrimental effects of each other. Overall however, the total time of aortic cross-clamping and fibrillation (approximately 30 min) equates to a significant myocardial ischaemic insult[313]. Several studies have compared clinical outcomes and the extent of myocardial injury in cross-clamp fibrillation and cardioplegia[314-316] and have found the two techniques to be comparable.

The profile of patients undergoing CABG surgery is changing over the years with increasingly higher-risk patients being operated upon[317]. The favourable metabolic effects of cardioplegia have encouraged more surgeons to adopt this technique in their practice although comparable clinical outcomes have been achieved with cross-clamp fibrillation[318].

Acute statin use as potential cardioprotective agent during CABG surgery

Despite the current cardioprotective techniques discussed above, myocardial injury still occurs and has been associated with adverse outcomes[158] [159] and as such there is a need for novel cardioprotective strategies. It is clear that treatment with HMG Co enzyme A reductase inhibitors ('statins') improves clinical outcomes in both patients at risk of coronary heart disease (CHD)[55] and in patients with established CHD[319]. Beyond improving lipid profiles, statins have 'pleiotropic effects' with actions, amongst others, on the vascular endothelium, platelet aggregation, oxidative stress, the stability of the atherosclerotic plaque and myocardial ischaemia-reperfusion (IR) injury[320]. Pre-clinical animal studies have demonstrated that the acute administration of 'statins' reduces myocardial injury, independently of their cholesterol lowering effects, when given prior to the index ischaemia[64] [77] [321] as well as at the point of reperfusion[77] [88] [89] [91]. With respect to its cardioprotective actions, the activation of the pro-survival kinases such as the PI3K-Akt and MEK1/2-Erk1/2, termed the Reperfusion Injury Salvage Kinase (RISK) pathway, is believed to underlie the protective action[53]. Interestingly, however, the chronic administration of 'atorvastatin' in animal studies failed to confer any cardio-protection, an effect which was attributed to the down-regulation of the PI3K-Akt component of the RISK pathway[78]. Of interest and potential clinical importance, the infarct-limiting effect of atorvastatin could be recaptured if a further high-dose of atorvastatin was administered acutely to these animals[78].

The objective of the current clinical study was to translate the above animal studies to the patient by determining whether the acute administration of high-dose atorvastatin was capable of reducing myocardial injury in patients, already on standard chronic 'statin' therapy, undergoing elective CABG surgery.

Methods

Study protocol

This study is one part of a larger group of studies entitled, "elucidating the mechanistic pathways of ischaemic preconditioning and postconditioning in the clinical setting" (see chapter 2). The full protocol was submitted for ethical approval to the joint University College London/University College London Hospitals Committee for ethical human research and sponsored by University College London Research and Development department. The trial was conducted according to University College of London Hospitals NHS Trust guidelines. In addition the patient information leaflet, consent form and general practitioner information leaflet were subject to ethical approval. Any amendments required to the study protocol were classed as minor or major according to criteria produced by the ethics committee. I made the amendments, and subject to the chief investigators approval, submitted the updated documents annotated with appropriate dates and version numbers for approval for each amendment. Any serious adverse event or reaction during the course of the study had to be reported to the R&D department as per adverse event guidelines. No serious adverse events or reactions occurred during the course of this study.

Patient population

We obtained informed written consent from all patients entered into the trial. Patients were approached on admission to hospital 24 hours prior to surgery and sufficient time and information was allowed to enable fully informed consent. Between July 2007 and January 2009 we recruited 45 consecutive adult patients to study 1 and 51 patients to study 2 who were due to undergo elective coronary artery bypass graft (CABG) surgery for severe coronary artery disease at our tertiary cardiac centre. The studies were performed sequentially with study 2 commenced following the results of study 1 and with a different dosing timing in order to explore whether a longer onset of action was required.

Inclusion criteria

All patients less than 80 years old undergoing elective 'on-pump' CABG surgery who were already established on a statin medication (atorvastatin, simvastatin, pravastatin or rosuvastatin) for more than four weeks and were able to give informed consent.

Exclusion criteria

We excluded patients over 80 years old; with known intolerance to 'statins'; with renal impairment (serum creatinine $> 120\mu mol/l$) or hepatic impairment (any abnormality in liver enzymes); with unstable angina or an acute myocardial infarction within 3 months or with angina within 3 days. Patients were excluded if taking glibenclamide.

Investigational Agent

Consenting patients were randomly assigned to either receive 160mg Atorvastatin 2 hours prior to surgery (study 1) or 12 hours prior to surgery (study 2) (figure 2) and 24 hours post-

operatively or their standard chronic 'statin' therapy. The anaesthetist and surgical team were blinded to the treatment allocation.

Surgical Procedure

All patients received an anaesthetic premedication of 10-20 mg of Temazepam 1 hour prior to surgery. On arrival in the anaesthetic room a peripheral venous cannula was inserted and an infusion of Hartmann's solution commenced. A radial arterial line was also inserted. Anaesthesia was induced using midazolam with or without propofol, fentanyl and pancuronium. Anaesthesia was maintained without the use of isoflurane and using a propofol infusion. The trachea was intubated and mechanical ventilation initiated. A central venous catheter was inserted, as was a nasopharyngeal temperature probe, transoesphageal echocardiographic probe and urinary catheter.

Figure 2: Schematic to show study design and patient recruitment.
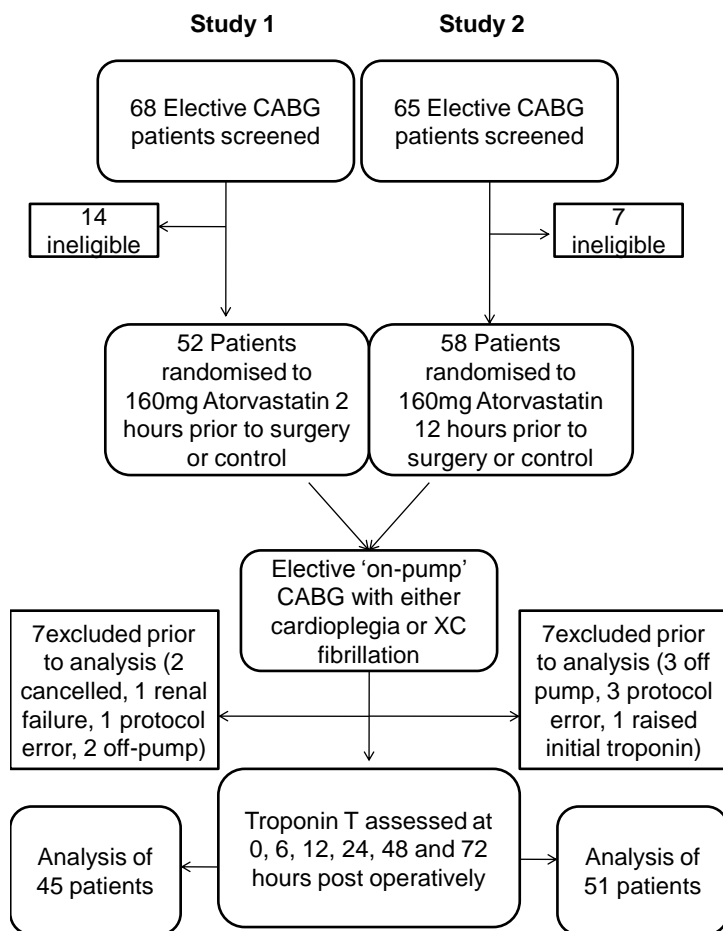
Cardiopulmonary bypass was standard, non-pulsatile with a membrane oxygenator and cardiotomy suction. All graft construction was done on cardiopulmonary bypass from aorta to target vessel or using the left internal mammary artery with either cold blood cardioplegia or intermittent cross-clamp fibrillation. Following construction of the grafts, cardiopulmonary bypass was discontinued and protamine used to reverse the heparinisation.

Blood samples for Troponin-T were taken prior to surgery and at 6, 12, 24, 48 and 72 hours following surgery. Quantitative Troponin T measurement was performed using a one-step immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche, Switzerland). The lower detection concentration of the assay was 0.01μg/L, with a recommended diagnostic range of 0.05-0.09 μg/L indicating possible myocardial injury and a

threshold of 0.1 µg/L or more indicating myocardial infarction. Internal quality control was performed on a daily basis with external quality control performed every 4 weeks.

Statistical Analysis

Data are presented as mean ± SD. Descriptive statistics were used to summarise the relevant features of the groups. Data was checked for normal distribution by plotting histograms with a normal curve. Comparison between treatment groups was made using the unpaired student T test. Difference between categorical data were analysed using the Chi Squared test. A value of P<0.05 was considered significant. A sample size of at least 45 patients was determined based on the following assumptions: (a) that from our previous studies we would expect a difference in total serum troponin-T release over 72 hours of approximately 15 µg/L between treated and untreated patients[322]; (b) a power of least 80%; (c) a standard deviation of 25 µg/L[322]; and (d) significance declared at the two-sided 5% level. After consent had been obtained for participation in the clinical trial, patients were randomly assigned to receive either atorvastatin treatment or control using a computer generated block randomisation sequence. The analysis was per-protocol, with this population defined by those patients who successfully received both doses of study medication, had a negative Troponin T at baseline, underwent CABG surgery with an 'on-pump' method and had at least 5 of 6 blood test results. The area under the curve indicative of absolute troponin release over 72 hours was calculated as follows:

$$AUC_{t1-t2} = [(TnT \text{ at } t1 \text{ hours} + TnT \text{ at } t2 \text{ hours}) / 2] \times (t2 - t1).$$

$$AUC72hours = AUC_{0-6} + AUC_{6-12} + AUC_{12-24} + AUC_{24-48} + AUC_{48-72}$$

Results

Study 1: 68 patients were assessed for eligibility and 52 patients fulfilled initial inclusion criteria and were recruited. 7 patients were excluded prior to analysis for not successfuly meeting study criteria for ongoing inclusion; 2 patients were cancelled at short notice after having had the first dose of study medication, 3 patients were operated using an 'off-pump' method, 1 patient developed renal failure requiring CVVHF making Troponin measurement unreliable and 1 patient received only 1 dose of the study medication. Study 2: 65 patients were assessed for eligibility and 58 fulfilled initial inclusion criteria and were recruited. 7 patients were excluded prior to analysis; 3 patients were operated using an 'off pump' method, 1 patient was found to have a raised Troponin T at baseline, 1 received only one dose of study medication and 2 patients had missing blood samples. (Figure 2).

There were no side effects from the atorvastatin noted either biochemically (peak CK over 72 hours post-operatively, study 1: Control 630 U/L, Atorvastatin 687 U/L, p=0.5, study 2: Control 658 U/L, Atorvastatin 819 U/L, p=0.3) or symptomatically in the intervention group. There were no significant differences between patient characteristics (see table 1 and 2).

Table 1: Patient characteristics

	Study 1		Study 2	
	Control (n=22)	Atorva (n=23)	Control (n=23)	Atorva (n=28)
Demographics Age (years)	61 ± 8.2	64 ± 8.0	63 ± 9.8	61 ± 10.8
Male	20 (91)	19 (83)	21 (91)	25 (89)
Female	2 (9)	4 (17)	2 (9)	3 (11)
Diabetes mellitus	7 (32)	4 (17)	6 (26)	6 (21)
Hypercholesterolaemia	20 (91)	19 (83)	17 (74)	21 (75)
Hypertension	14 (64)	18 (78)	17 (74)	16 (57)
Previous myocardial infarction	6 (27)	7 (30)	9 (40)	7 (25)
Previous stroke	0 (0)	0 (0)	0 (0)	0 (0)
Peripheral vascular disease	2 (9)	0 (0)	2 (9)	1 (4)
Current smokers	4 (18)	2 (9)	1 (4)	3 (11)
Ex-smokers	8 (36)	6 (26)	13 (57)	15 (57)
Never smoked	10 (45)	15 (65)	9 (39)	10 (36)
Family history of IHD	7 (32)	13 (57)	12 (52)	14 (50)
Body Mass Index	28 ± 4.2	29 ± 2.8	29 ± 4.6	30 ± 4.6
NYHA class	1.8 ± 0.7	1.8 ± 0.8	1.5 ± 0.6	1.9 ± 0.6
CCS class	1.6 ± 0.8	1.8 ± 0.9	1.5 ± 0.8	1.9 ± 0.7
Ejection fraction >55%	20 (91)	20 (87)	19 (83)	25 (89)
35-55%	2 (9)	1 (4)	3 (13)	2 (7)
<35%	0 (0)	2 (9)	1 (4)	1 (4)
Euroscore	2.8 ± 1.7	2.9 ± 2.7	2.16 ± 1.7	1.85 ± 1.9

	Study 1		Study 2	
	Control (n=22)	Atorva (n=23)	Control (n=23)	Atorva (n=28)
Drug history				
Aspirin	0 (0)	5 (22)	2	2 (7)
Beta-blocker	14 (64)	20 (87)	17 (74)	27 (96)
Statin	22 (100)	23 (100)	23 (100)	28 (100)
ACE-inhibitor/ACE antagonist	13 (59)	18 (78)	15 (65)	16 (57)
Long-acting nitrates	7 (32)	4 (17)	5 (22)	6 (21)
Calcium Channel Antagonist	8 (36)	5 (22)	9 (39)	4 (14)
Nicorandil	2 (9)	2 (9)	0 (0)	1 (3)
Anti-diabetic drugs				
Insulin	1 (5)	2 (9)	0 (0)	1 (4)
Sulphonylurea	1 (5)	0 (0)	5 (22)	2 (7)
Metformin	2 (9)	3 (13)	3 (13)	4 (14)

Values presented as mean ± SD or 'N' (%)

Table 2: Details of cardiac bypass surgery

	Study 1		Study 2	
	Control	Atorva	Control	Atorva
Bypass-time (min)	80 ± 23	84 ± 39	83 ± 24	80 ± 21
Cross-clamp time (min)	45 ± 16	48 ± 25	49 ± 15	48 ± 13
Intermittent cross-clamp fibrillation	6 (27)	9 (39)	2 (9)	4 (14)
Cardioplegia	16 (73)	14 (61)	21 (91)	24 (86)
Number of grafts:				
One	0 (0)	1 (4)	0 (0)	0 (0)
Two	6 (27)	2 (9)	0 (0)	4 (14)
Three	12 (55)	12 (52)	16 (70)	18 (64)
Four	3 (14)	8 (35)	6 (26)	6 (21)
Five	1 (5)	0 (0)	1 (4)	0 (0)

Values presented as mean ± SD or 'N' (%)

There was no significant difference in serum Troponin T at each time point and total Troponin release over 72 hours, calculated as the Area Under the Curve (AUC). Study 1: Atorvastatin 29.6±34.8 µg/L, Control 25.0±22.0 µg/L (95% CI -13.0 to 22.2, P=0.6). Study 2: Atorvastatin 21.8±14.3 µg/L, Control 20.9±8. µg/L (95% CI -5.9 to 7.7, P=0.8) (See table 3, figure 3 and figure 4).

Table 3: Serum troponin-T levels over the 72 hours following CABG surgery

	Time after surgery (hrs)	0	6	12	24	48	72
Study1	Control	0	0.80 ± 0.55	0.61 ± 0.42	0.37 ± 0.35	0.31 ± 0.29	0.24 ± 0.26
	Atorva	0	0.82 ± 0.51	0.58 ± 0.38	0.39 ± 0.38	0.42 ± 0.77	0.30 ± 0.41
	Mean Difference	0	0.20 ± 0.16	-0.32 ± 0.12	0.02 ± 0.11	0.11 ± 0.18	0.06 ± 0.11
	Confidence Interval	-	-0.31 to 0.35	-0.28 to 0.22	-0.20 to 0.24	-0.26 to 0.48	-0.17 to 0.29
	P value	-	P=0.9	P=0.8	P=0.9	P=0.5	P=0.6
Study 2	Control	0	0.66 ± 0.33	0.50 ± 0.23	0.27 ± 0.10	0.22 ± 0.10	0.20 ± 0.10
	Atorva	0	0.64 ± 0.39	0.45 ± 0.28	0.29 ± 0.22	0.25 ± 0.17	0.27 ± 0.22
	Mean Difference	0	-0.17 ± 0.10	-0.04 ± 0.07	0.02 ± 0.05	0.02 ± 0.04	0.06 ± 0.05
	Confidence Interval	-	-0.23 to 0.19	-0.19 to 0.10	-0.08 to 0.12	-0.06 to 0.10	-0.04 to 0.17
	P value	-	P=0.87	P=0.56	P=0.66	P=0.58	P=0.23

Values presented as mean ± SD (in µg/L)

Figure 3: Shows the mean cardiac Troponin T release over 72 hours following elective CABG surgery in control and atorvastatin treated groups in study 1 (treated 2 hours pre-operatively) and study 2 (treated 12 hours pre-operatively).
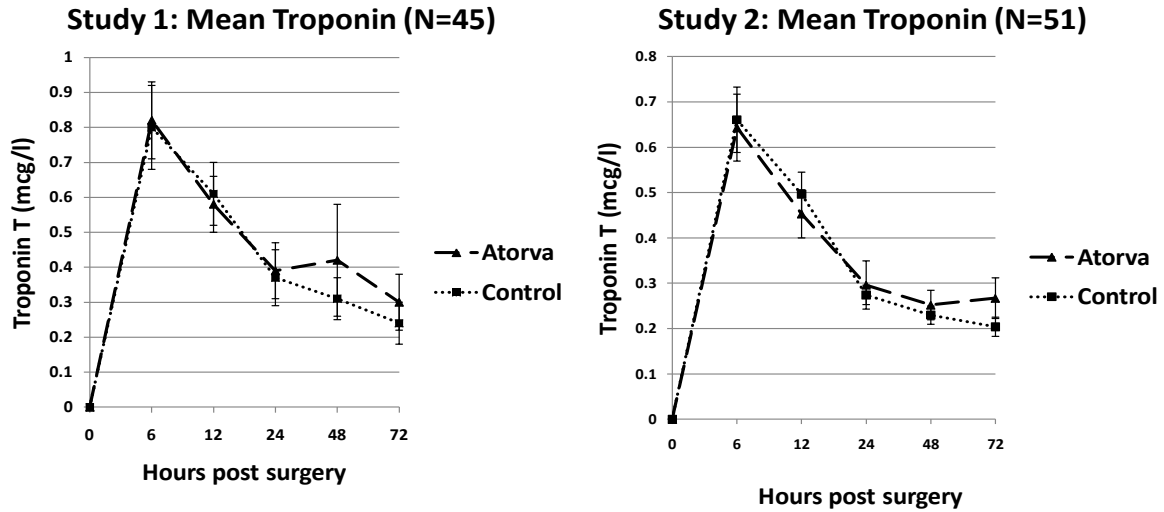
Figure 4: The mean cardiac Troponin T AUC over 72 hours post-operatively in study 1 and study 2.
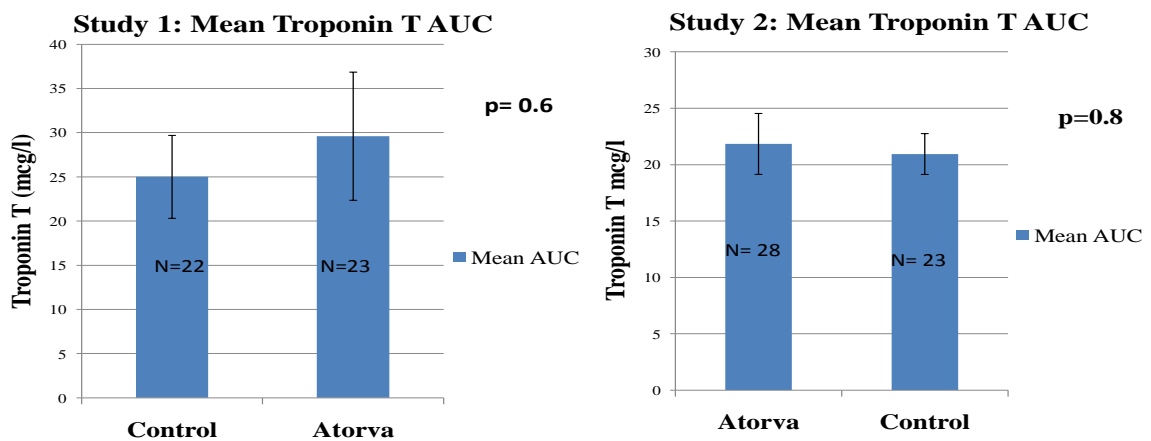
There was no significant difference in the number of patients with a Troponin-T ≥ 0.46 µg/L at 48 hours post-operatively, which is reported to correlate to a worse prognosis[158], in either study (study 1: Control- 3 patients, Atorvastatin- 3 patients; study 2: Control- 0 patients, Atorvastatin- 4 patients).

There was no significant difference in the relative distribution of size of Total Trop-T AUC over the 72 hours post-operatively (see table 4 and figure 5).

Table 4: Distribution of Total Trop T AUC over 72 hours from studies 1 and 2

		Percentage of study patients with Total Trop T AUC over 72 hours (µg/L) in the categories below				
		0 to 9.9	10 to 19.9	20 to 29.9	30 to 39.9	≥40
Study 1	Control(%)	13.6	36.3	27.3	9.1	13.6
	Atorva(%)	13.0	30.4	26.1	21.7	8.7
Study 2	Control(%)	8.7	47.8	21.7	21.7	0
	Atorva(%)	10.7	35.7	35.7	7.1	10.7

The current consensus guidelines on the definition of MI define a peri-operative MI following cardiac surgery as requiring elevation of cardiac enzymes greater than 5 times the ULN with new Q waves on ECG, angiographic evidence of new coronary artery occlusion or

non-invasive imaging suggesting newly non-viable myocardium[111]. Although in these studies ECG and imaging data was not obtained, an analysis of the incidence of patients in each group with a peak Troponin-T > 5 times the ULN showed no difference (Study 1: Atorva 74% vs Control 73%; p=ns. Study 2: Atorva 57% vs Control 61%; p=ns). We do not have enough evidence to label these patients as having had a peri-operative myocardial infarction, a considerable number of patients do however have substantial measured Troponin release following their surgery although this is equal between the groups.

An analysis of Trop-T AUC over 72 hours within studies 1 and 2 by intra-operative cardioprotective technique showed that patients operated with a cross-clamp fibrillation method displayed a non-significant trend towards less myocardial injury although the numbers of patients in the cross-clamp fibrillation group were relatively small. There was no significant difference between atorvastatin treated and control patients. (See figure 6).

Figure 5: Graph to show the percentage of patients against the relative size of the Total Troponin-T AUC over 72 hours in studies 1 and 2.
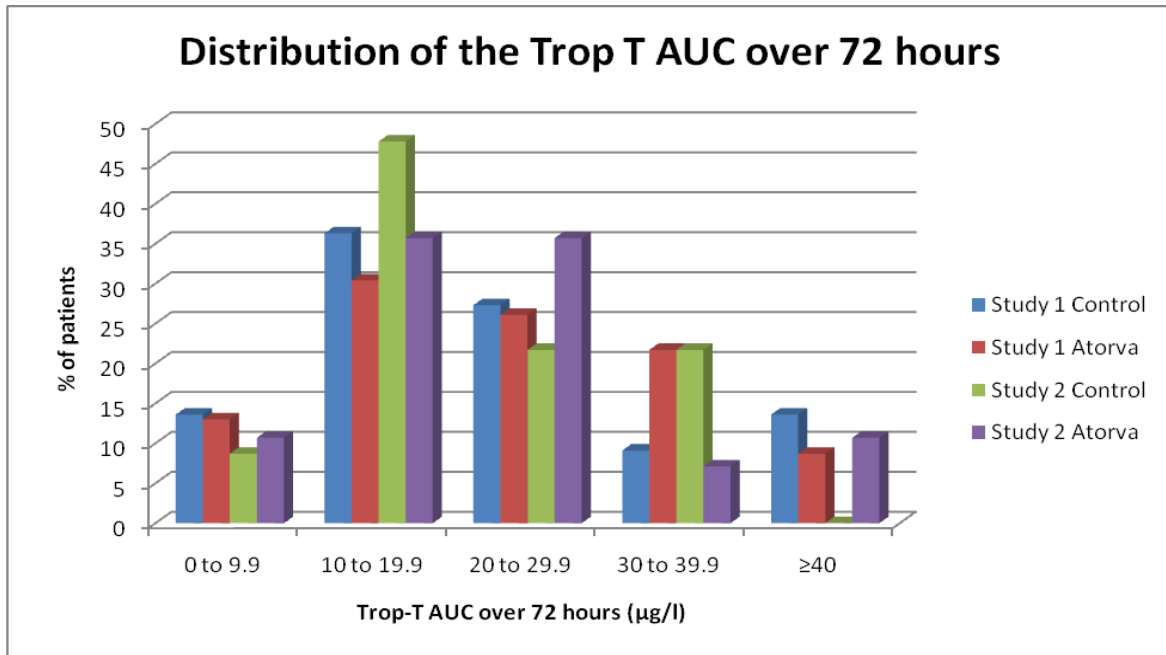
Figure 6: Graph to show the Trop-T AUC over 72 hours by intra-operative cardioprotective technique.
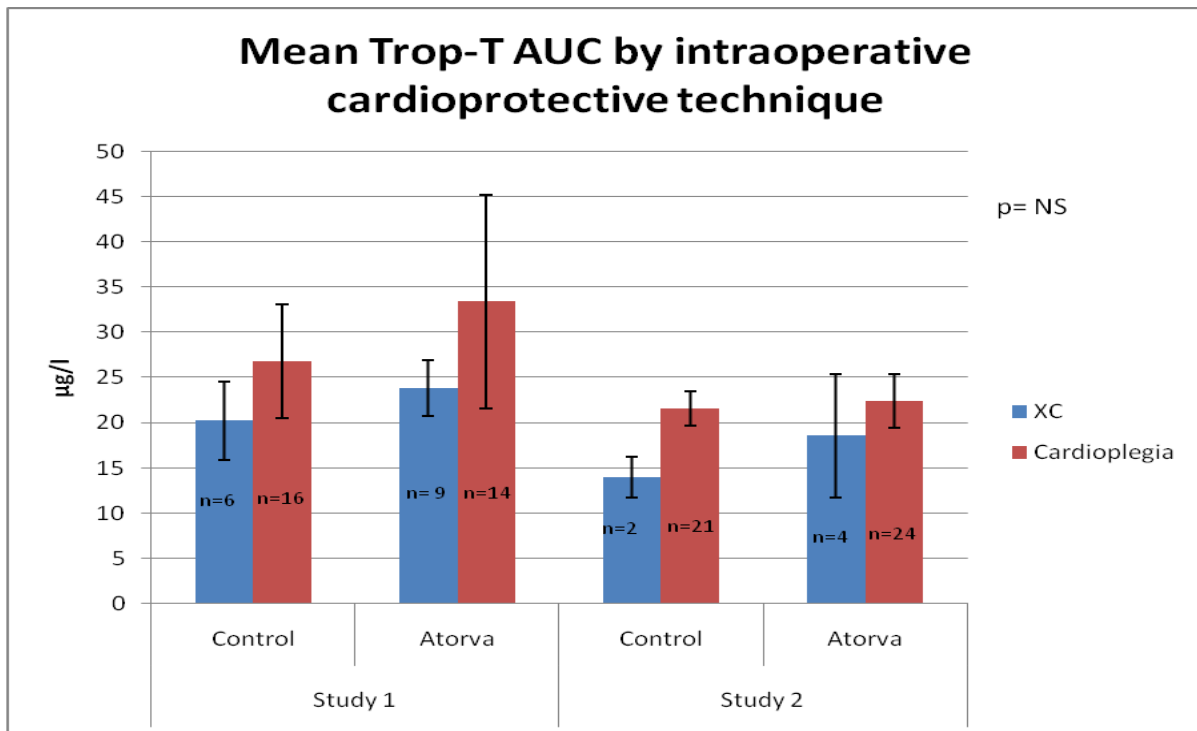
Discussion

From the results of these randomised controlled single blinded clinical studies this thesis reports that high-dose atorvastatin administered acutely to patients already on chronic standard 'statin' therapy either 2 or 12 hours pre-operatively is safe but did not reduce myocardial injury during elective CABG surgery. This was evidenced by the lack of difference in cardiac enzyme release in patients randomised to receive the high-dose atorvastatin treatment when compared to standard 'statin' therapy. On post-hoc sub-group analysis there was no difference in cardiac enzyme release between patients operated using cardioplegia or cross-clamp fibrillation cardioprotective techniques. There was no difference either in mean peak Troponin release, in number of patients with a peak Troponin greater than 5 times the upper limit of normal during the 72 hours post-operatively or in the incidence of patients with a Trop-T >0.46μg/l at 48 hours post-op which has been linked to adverse outcomes.

In the clinical setting, 'statins' have been demonstrated to exert beneficial effects beyond that expected from cholesterol lowering. In the MIRACL study, the early administration of high-dose atorvastatin in the first few days following an acute coronary syndrome reduced recurrent symptomatic ischaemia and subsequent hospitalisation[138]. In 'statin' naïve patients, atorvastatin has been reported to reduce peri-procedural myocardial injury in both patients undergoing elective PCI[119] and in NSTEMI patients undergoing PCI[128] (for a comprehensive review see[323]).

However, the beneficial effect of 'statin' use prior to CABG surgery is somewhat less clear; a retrospective analysis of 5,439 patients undergoing elective CABG surgery found no effect of chronic administration of 'statins' on clinical outcomes[164]. However a prospective observational study of 5,436 patients undergoing elective CABG surgery did show

significantly improved early all-cause mortality in patients taking chronic 'statin' therapy, but there was no improvement in the incidence of peri-operative myocardial infarction[165]. Statin medication commenced following CABG surgery has been associated with an improved mortality and reduced cardiac events at 6 months[324]. Recently a randomised study of rosuvastatin versus placebo in a selected population of patients undergoing CABG was performed. Patients treated for 7 days prior to CABG with rosuvastatin showed significantly less myocardial injury perioperatively[169]. However, in that particular study, patients already taking a statin were required to stop treatment 30 days prior to the study protocol and all patients were only treated for 7 days pre-operatively and for an unstated post-operative period; this therefore does not reflect common clinical practice. High doses of acute statins have not been shown to reduce the burden of atrial fibrillation following CABG surgery[325].

Experimental studies using mice have clearly demonstrated that the acute administration of 'statins' can reduce myocardial infarct size by approximately 50%. For example, treatment with acute atorvastatin at the onset of myocardial reperfusion following a period of lethal ischaemia reduced myocardial infarct size by approximately 50% in a manner dependent on PI3K, Akt and the upregulation of eNOS[88]. Crucially, the chronic administration of standard dose atorvastatin did not reduce myocardial infarct size. The failure to demonstrate cardioprotection was attributed to the upregulation of phosphatase tensin homolog deleted on chromosome ten (PTEN), a phosphatase which down-regulates the phosphorylation of Akt, a key mediator of cardioprotection. However, the re-activation of Akt using a high-dose of atorvastatin was able to recapture the cardioprotective effect of atorvastatin in these mice treated with chronic standard atorvastatin therapy[78]. The mechanism of action for the reactivation of the cardioprotective effect is not known. It has been speculated that this increased dose may activate other pathways which are cardioprotective such as the

extracellular signal-related kinase 1/2 (ERK 1/2)[46] or whether it is just a matter of a higher dose being needed to overcome the upregulated PTEN in order to then activate the RISK pathway. The theory that cardioprotection can be 'recaptured' has been tested in the setting of PCI in the ARMYDA-Recapture study. 352 patients already established on statin medication were randomised to a 'reload' of 80mg atorvastatin 12 hours pre-PCI and 40mg immediately pre-PCI or placebo. Peri-procedural injury was significantly reduced[123].

In the current study, we were interested to determine whether the acute administration of a high-dose of atorvastatin could elicit cardioprotection in adult CABG patients already on standard dose 'statin' therapy, however we did not observe any reduction in myocardial injury which is in contrast to the findings of the ARMYDA-Recapture study. The ARMYDA group have demonstrated that additional cardioprotection can be afforded by further acute dosing of atorvastatin. They report that this also reduces the incidence of 30 day MACE although all such events were peri-procedural. It is possible that the setting of PCI is more amenable to the study of this effect as there are less complicating factors not only in terms of causes of myocardial injury but also in regards to the much more invasive nature of surgery and the CPB required. Also, the translation of findings made in laboratory animal studies into the clinical environment may be hampered by several factors (reviewed in [326]). The study of atorvastatin as a cardioprotective agent has for the most part been restricted to non-atherosclerotic juvenile animal models of experimental myocardial infarction, in the absence of co-morbid illnesses and other medication. Patients undergoing CABG surgery are older, often have severe atherosclerotic coronary artery disease, are on various medications, have other co-morbidities and so on. Furthermore it has recently been shown that aspirin may abrogate the protective effect of atorvastatin in ischaemia-reperfusion injury[327] and although the majority of patients had ceased their aspirin prior to surgery it is not known whether the

effect was still present. Work from the same group has also shown that a low dose of atorvastatin, which alone does not cardio-protect, can show protection when combined with other agents such as dipyridamole[82] and sildenafil[328] and future clinical work, potentially combining atorvastatin with these agents, may well yield more positive results. Furthermore, many of the experimental studies demonstrating cardioprotection with statin administration have used animal models of coronary artery occlusion, but pre-clinical investigation of statin therapy using an animal cardiac bypass model of ischaemia-reperfusion injury has been lacking.

The myocardial injury encountered during CABG surgery comprises in addition to ischaemia-reperfusion, coronary microembolisation and direct myocardial injury from surgical handling. However, several clinical studies have reported that the myocardial injury sustained during CABG surgery is associated with worse clinical outcomes, and the release of cardiac-specific enzymes can be used to stratify patient outcome[157][158]. Indeed, using this clinical environment, we have recently been able to demonstrate the efficacy of remote ischaemic preconditioning using transient ischaemia of the arm in terms of a significant reduction in myocardial injury as measured by the 72 hour peri-operative release of serum troponin T[322][329] although it must be appreciated that this has been replicated by some groups[330] but not others[331]. We believe that this confirms that the setting of CABG surgery is indeed an appropriate one in which to investigate the cardioprotective potential of high-dose atorvastatin therapy.

We chose 160mg of atorvastatin as the acute dose to be administered because this was almost certainly going to be significantly higher than any patient's current dose. This dose has previously been found to be safe[332] and has been shown to produce comparable serum blood levels in humans to the doses used in animal experiments[333]. We found this dose to be

well tolerated with no recorded adverse effects. The acute dose of atorvastatin was given 2 hours or 12 hours prior to surgery and then 24 hours following surgery. The peak plasma concentration of atorvastatin occurs between 1-2 hours after administration[334] and although absorption can be affected by food[335] all our patients were fasted prior to surgery. The first study administered atorvastatin 2 hours prior to surgery on the basis that peak serum levels occurred at this point. However once the results were analysed and discussed the possibility was raised that peak action may be somewhat later than peak serum levels. Hence the second study was performed with atorvastatin administered 12 hours prior to surgery.

In summary, this thesis reports that the acute administration of high dose atorvastatin to adult, low risk patients already on a standard statin dose regimen was safe but did not reduce myocardial injury encountered during elective CABG surgery. It remains a possibility that patients at higher risk of intraoperative myocardial injury may have the potential to be protected and this warrants further investigation.

CHAPTER 4

Effect of erythropoietin as an adjunct to primary percutaneous coronary intervention: a randomised controlled clinical trial

Introduction

Primary percutaneous coronary intervention (PPCI) is now established as the superior method of reperfusion in ST Elevation Myocardial Infarction (STEMI)[336], however despite this recent advance the morbidity and mortality of STEMI remains significant. This may be due, in part, to myocardial reperfusion injury, a phenomenon in which the reperfusion of ischemic myocardium induces further cardiomyocyte death, thereby limiting the beneficial effects of PPCI[7]. Therefore, novel cardioprotective agents capable of reducing myocardial reperfusion injury, are required to realise the full benefits of reperfusion therapy, limit myocardial infarct size, preserve cardiac function and improve clinical outcomes in these patients.

There is considerable evidence that the cytokine, erythropoietin (EPO), exerts pleiotropic effects beyond that of haematopoiesis, which include both neuroprotection and cardioprotection[337]. The acute administration of high-dose EPO at the time of reperfusion has been reported in animal models to reduce myocardial infarct size by approximately 50%[337]. Whether EPO is beneficial in STEMI patients when administered as an adjunct to PPCI is unknown and is investigated in this proof-of-concept randomised clinical trial.

Methods

Study Protocol

This study is one part of a larger group of studies titled, "elucidating the mechanistic pathways of ischaemic preconditioning and postconditioning in the clinical setting" (see chapter 3, figure 1). The full protocol was submitted for ethical approval to the joint University College London/University College London Hospitals Committee for ethical human research and sponsored by University College London Research and Development department. The trial was conducted according to University College of London Hospitals NHS Trust guidelines. In addition the patient information leaflet, consent form and general practitioner information leaflet were subject to ethical approval. Any amendments required to the study protocol were classed as minor or major according to criteria produced by the ethics committee. I made the amendments, and subject to the chief investigators approval, submitted the updated documents annotated with appropriate dates and version numbers for approval for each amendment. Any serious adverse event or reaction during the course of the study had to be reported to the R&D department as per adverse event guidelines. The study was registered with the United Kingdom Clinical Research Network (Study ID 4058).

Patient selection

Between July 2007 and August 2009 we conducted a randomised double-blind placebo-controlled trial of STEMI patients referred to a single tertiary cardiac centre for PPCI (see figure 1). We obtained written informed consent from all patients who entered the study (for a discussion on informed consent in research in STEMI see appendix 2).

Inclusion criteria

Patients were included if they presented for PPCI within 12 hours of onset of symptoms and had the following ECG criteria ($\geq$2mm ST-elevation in chest ECG leads, $\geq$1mm ST-elevation in contiguous limb leads or new onset left bundle branch block), were under 80 years old with a single vessel culprit lesion, and had Thrombolysis in Myocardial Infarction (TIMI)[338] grade 0 and Rentrop[339] collateral grade 0 on coronary angiography.

Figure 1: Schematic to show patient recruitment and study flow.
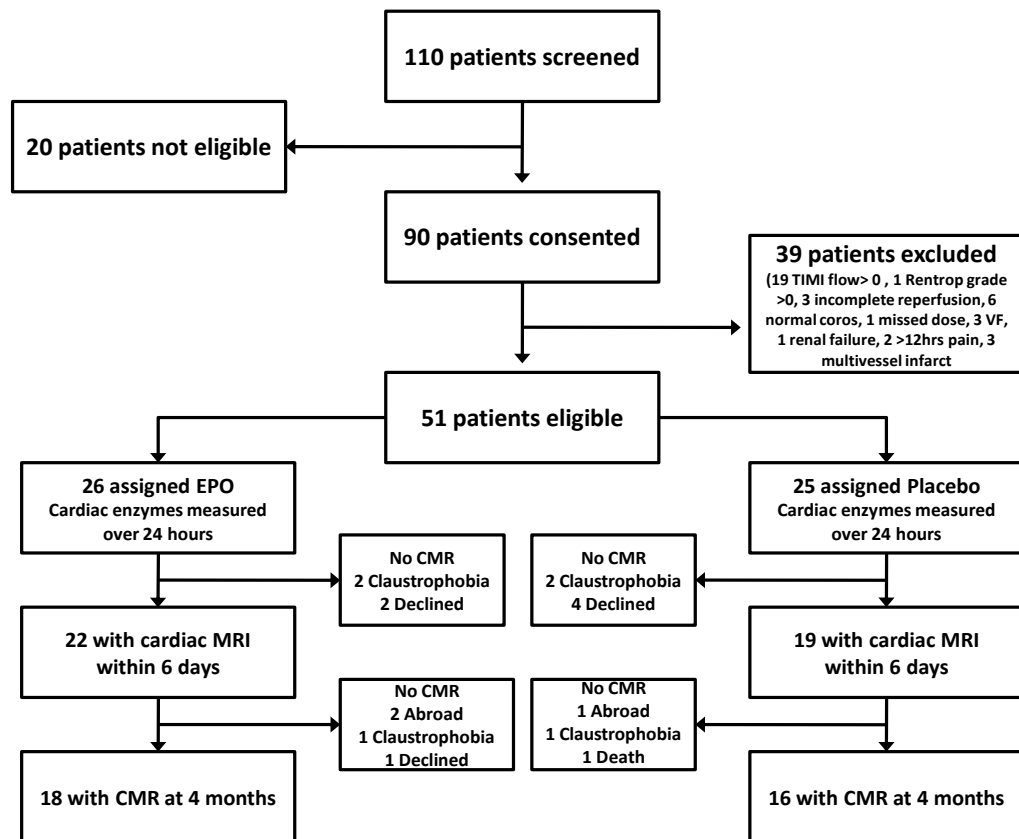
Exclusion Criteria

Exclusion criteria included abnormal renal function (serum creatinine >120 µmol/L), known thromboembolic disorder, malignant disease, multi-vessel disease, failure to achieve TIMI 3

flow at the end of the PCI procedure, cardiogenic shock and cardiac arrest. Patients were excluded from CMR if they had any metal implants which rendered CMR unsafe or if they were claustrophobic.

Procedures

Potentially eligible patients were approached at their acute admission with their STEMI. Due to the time pressures involved of this emergency treatment a rapid assessment of preliminary eligibility was made and consent taken. However entry in to the trial was not confirmed until following the PPCI and the patient had fulfilled the pre-specified inclusion criteria and did not meet any excludion criteria, as above. Patients were randomly allocated to receive either EPO treatment or placebo prior to PPCI. A computer-generated blocked randomisation list was prepared in advance of the trial. Treatment allocation was undertaken by a Clinical Research Fellow (not involved with assessing clinical outcomes or the PPCI procedure) using sealed opaque envelopes. The patient, the PPCI operator, and the assessor of clinical outcomes were blinded to the treatment allocation.

Investigational agent

The EPO treatment arm comprised 50,000 IU of rhEPOβ (Neorecormon, Roche Ltd., Welwyn Garden City, UK) in 10 mls 0.9% saline, whereas the placebo comprised 10 mls 0.9% saline. Either was administered via a peripheral intravenous cannula over 2 minutes prior to PPCI, with an additional rhEPOβ dose or placebo administered via the same route 24 hours following PPCI. Once reconstituted the solutions were identical. This dose of rhEPOβ was obtained from a previously published proof-of-concept clinical study which suggested that rhEPOβ (33,000 IU) administered daily for 3 days (total dose 99,000 IU) may be

beneficial in stroke patients[240]. We used this total dose but over 24 hours to ensure no barrier to early discharge if indicated on day 2.

Percutaneous Coronary Intervention

PPCI was undertaken according to the Cardiologists' preference with no restriction placed on vascular access route, type of stent or method of stenting (predilatation or direct). Bystander PCI was permitted as long as this was not a second culprit vessel. However, thrombolytics and adenosine were not used.

Study end-points

Blood samples for Troponin-T and CK-MB were taken prior to PPCI and 6, 12, 24 hours post-procedure. Blood samples for haemoglobin, haematocrit, platelet count, prothrombin time, and renal function were taken at baseline and at 2 days post-PPCI.

The primary end-point was myocardial infarct size, determined by the 24 hour area-under-curve (AUC) serum Troponin-T. Secondary end-points included 24 hour AUC serum CK-MB, and CMR-determined myocardial infarct size, myocardial salvage index, LV volumes, mass and ejection fraction, and the presence of MVO. All in-hospital major adverse cardiac and cerebrovascular events (MACCE) were recorded and patients undergoing CMR at 4 months were interviewed for any MACCE during the follow up period.

CMR imaging

CMR imaging was performed at a median of 2 days from PPCI (range 1 to 6 days) and repeated at 4 months using a 1.5-T scanner (Avanto-Siemens, Erlangen, Germany). Patients

were transferred (using a critical care ambulance service with medical escort) to Great Ormond Street Hospital for CMR to take place as at the time of the study there was no CMR scanner on site at The Heart Hospital. Left ventricular (LV) function and volumes were assessed by standard steady-state free precession technique. Consecutive short-axis views were obtained by encompassing the LV from base to apex; vertical and horizontal long-axis views were acquired. Typical image parameters were as follows: TE 1.16ms, TR 2.73ms, flip-angle 65°, matrix 144x192, slice-thickness 7mm, gap 3mm. A velocity encoded aortic flow map was acquired to confirm LV volumes. Rest myocardial perfusion images were evaluated with a first-pass technique using a T1-weighted multi-shot gradient echo-planar inversion-recovery sequence (TR 2.6ms, TE 1.1ms, TI 200ms, flip-angle 12°, slice-thickness 10mm). Three short-axis slices (basal, mid-cavity, and apex) were obtained injecting 0.2mmol/kg gadolinium (Dotarem®; Guerbet SA, Paris, France) at 2ml/s followed by a 20ml saline flush into an antecubital vein. Early gadolinium enhancement (EGE) images were acquired 1-2 minutes after gadolinium injection with a fixed inversion time (TI) of 440ms. Two-dimensional slices in LV short axis were imaged with no inter-slice gap. (TR 7.0ms, TE 4.9ms, flip-angle 23°). Late gadolinium enhancement (LGE) images were acquired in long- and short-axis views with a segmented inversion-recovery fast gradient echo sequence 10 minutes after the contrast injection. Sequence parameters were as follows: TR 8.9ms, TE 4.9ms, flip-angle 25°, slice-thickness 7mm, gap 3mm. The inversion time was optimised to null normal myocardium.

CMR analysis

All CMR images were analysed by an experienced CMR reader (A.L. or J.H.). All LV volume data were independently and blindly analysed by both readers. LV ejection fraction, end-systolic and end-diastolic volumes and mass were calculated from segmentation of the

LV and indexed to body-surface-area (see figure 2). The EGE images were assessed qualitatively for the presence or absence of microvascular obstruction (MVO) (see figure 3). Myocardial infarct mass was measured by manual segmentation of areas of LGE and analysed by concordance between the two readers. In case of discordance, blinded review by a level III accredited CMR reader (J.M. or D.J.H.) was performed. Analysis was performed using Osirix (version 3.5.1) software. Inter-observer variability was calculated (table 4).

Figure 2: Example of LV segmentation analysis.
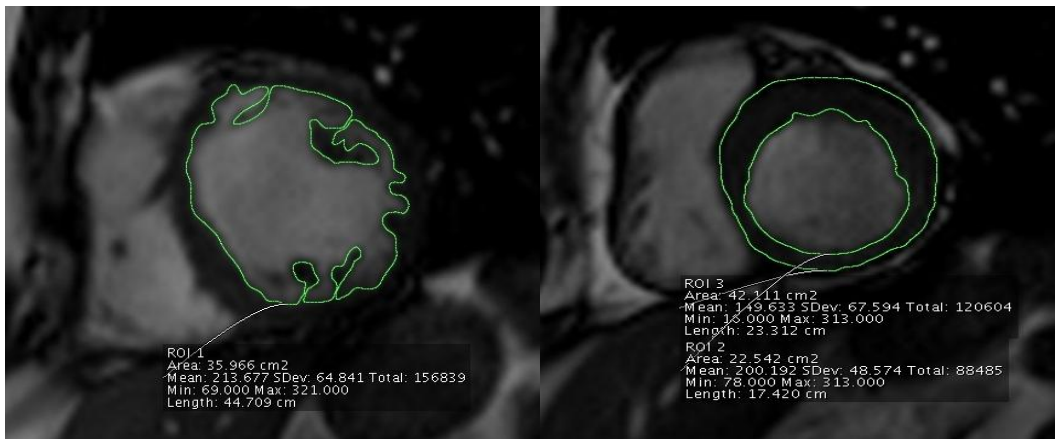
The figure shows one slice in short axis through the left ventricle at end diastole (left panel) and end systole (right panel) with manual traces around the endocardial and epicardial borders. This is repeated throughout the ventricle allowing total cavity and myocardial volumes to be calculated

Figure 3: Representative images of gadolinium enhancement from an initial CMR scan.
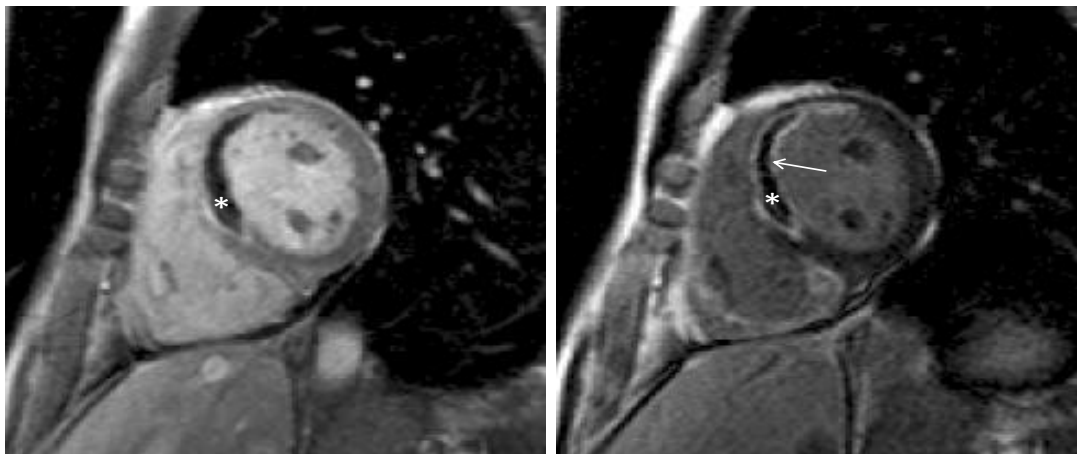
The left panel displays early gadolinium enhancement revealing evidence of microvascular obstruction (*) and the right panel, late gadolinium enhancement depicting a transmural myocardial infarct (arrow) with a core of microvascular obstruction (*).

Determining the myocardial salvage index

When assessing the efficacy of a reperfusion treatment strategy, it is essential to express myocardial infarct size (IS) as a percentage of the area-at-risk (AAR). In this study, the AAR was quantified using both coronary angiography (modified Bypass Angioplasty Revascularisation Investigation [BARI][273] (figure 4) and modified Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease [APPROACH] (figure 5) jeopardy scores)[299] and the acute CMR scan (infarct endocardial-surface-area [Infarct-ESA](figure 6))[299]. As a measure of the AAR, infarct-ESA has been validated against the BARI and modified APPROACH scores[299] and T2-weighted imaging of myocardial oedema[340]. Myocardial salvage index was calculated as follows (AAR-IS)/AAR.

Figure 4: Diagram to show the BARI angiographic myocardial risk score[273] and how it may be applied to a right coronary artery occlusion.
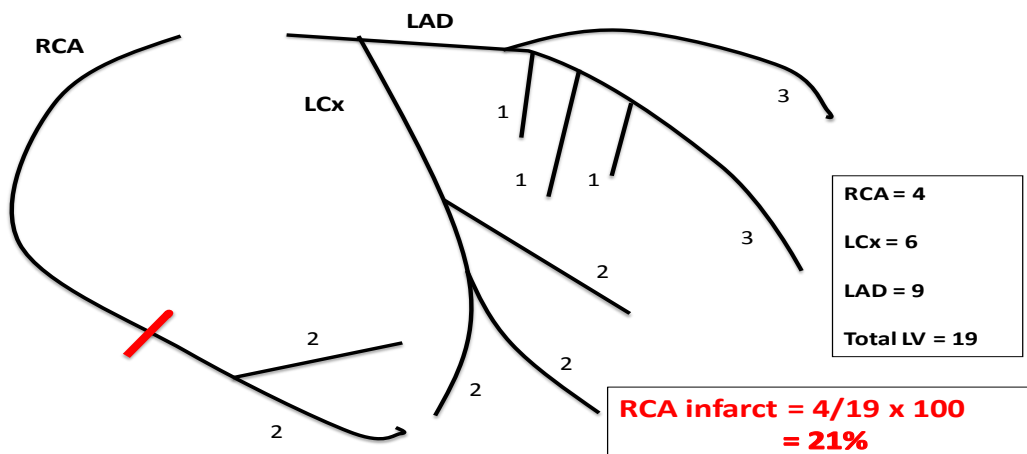
RCA- right coronary artery, LCx- left circumflex artery, LAD- left anterior descending, LV- left ventricle.

Figure 5: The modified- Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease (APPROACH) angiographic myocardial risk score.[299]

Culprit lesion location	Infarct related artery side branches ↓	→	Diagonal for LAD occlusion only Or Posterolateral for all others		
			Small or absent	Medium	Large
LAD (RD or LD)		Distal	13.75	14.8	15.9
		Mid	27.5	29.7	31.8
		Proximal	41.25	44.5	47.75
Proximal LCx (RD)	OM	Small or absent	9.25	12.5	15.75
		Medium	15.25	18.5	21.75
		Large	21.25	24.5	27.75
Proximal LCx (LD)	PDA	Small or absent	23.5	28	32.5
		Medium	29.5	34	38.5
		Large	35.5	40	44.5
Mid LCx (LD) or RCA (RD)	PDA	Small or absent	9.25	12.5	15.75
		Medium	15.25	18.5	21.75
		Large	21.25	24.5	27.75
Mid LCx (RD)			3.25	6.5	9.75

LAD-left anterior descending, RCA- right coronary artery, LCx- left circumflex artery, PDA-posterior descending artery, LD- left dominance, RD- right dominance.

Figure 6: The infarct- endocardial surface area (infarct-ESA) method of measuring myocardial area at risk.[299]
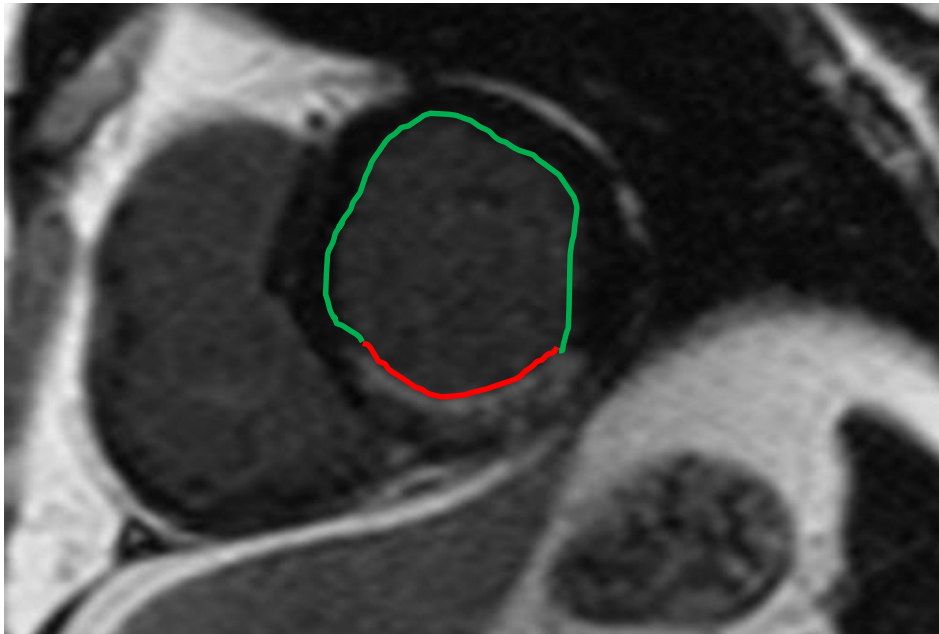
The endocardial surface area of the infarct (red) is traced on each slice through the left ventricle in short axis and expressed as a percentage of the total LV endocardial surface area (red and green) which gives a percentage of LV myocardium at risk.

Statistical analysis

The patient cohort was made up of those patients who fulfilled all study entry criteria at the end of their PPCI procedure and successfully received the correct study protocol. Descriptive statistics were computed to summarise the relevant features of the data. An unpaired t-test was used to test the differences in means in the EPO versus placebo groups for continuous data and 95% confidence intervals were calculated for the difference of the means between the EPO and placebo groups. The assumptions of the t-test were examined using residuals analysis. A sensitivity analysis was performed where individual patients had Studentised residuals greater than two standard deviations by omitting these patients from the analysis and examining the impact to the mean difference and 95% confidence intervals. A Bland-

Altman[341] plot was used to calculate the difference and limits of agreement between the two observers for each continuous clinical measurement. The number of measurements falling outside the limits of agreement was used as a heuristic guide to agreement. A paired t-test of the measurements was used to formally test that the difference of the means of the two measurements was zero. In addition, the reliability (intra-class correlation) was computed for each continuous clinical measurement. A kappa statistic was calculated to measure the observer agreement for categorical clinical measurements. For categorical data a Z-test of proportions was used to test the equality of the proportion of patients in the EPO group versus the control group. An approximate 95% confidence interval for the difference in the proportions in the two groups was also calculated.

At study inception there were no similar studies that had used Troponin-T as an end point or reduction in myocardial infarct size as measured by cardiac MRI and therefore gauging treatment effect in order to estimate sample size was based on the successful study of ischaemic post-conditioning in acute myocardial infarction[30]. The sample size was therefore calculated from the effect on total CK from this study with the presumption that Troponin release would be similarly affected. A sample size of at least 44 patients in total was determined based on the following assumptions: (a) A 36% reduction in total Troponin T release (b) power of at least 80%; (c) a standard deviation of 40%; and (d) significance declared at the two-sided 5% level. All analyses were done with Stata version 11 or SPSS statistical software version 15.0.

Results

110 patients were screened for inclusion in the study with 20 patients immediately recognised as ineligible. At this stage 90 patients were consented for the study prior to coronary angiography. Following angiography a further 39 patients were excluded as more criteria for

exclusion were identified. Therefore 51 patients were successfully randomised, to receive either placebo (n=25) or rhEPOβ (n=26) (figure 1). The treatment groups were well balanced, apart from a non-statistically significant predominance of LAD infarcts in the placebo group, and the characteristics are summarised in tables 1 and 2. There was good agreement using the three different scores to measure the area-at-risk. Table 3 summarises the AAR scores and the main results. Myocardial infarct size assessed by 24 hour AUC serum Troponin T and CK-MB (figure 7) and by the initial and follow-up CMR scan showed a non-significant increase in the EPO-treated group compared to placebo. Unexpectedly, at the initial CMR scan, the presence of microvascular obstruction was almost doubled in the EPO-treated group when compared to placebo. Furthermore, there was an increase in indexed LV end-systolic and end-diastolic volumes and mass in the EPO-treated group compared to placebo (figure 8 and 9). There were no significant differences between the groups in terms of the LV stroke volume index and LV ejection fraction and the myocardial salvage index. There were no significant differences in the CMR end-points on the 4 month follow-up scan, although there

Table 1. Patient characteristics

	EPO (n=26)	Placebo (n=25)
Age, mean yrs	55.5 (12.8)	61 (10.0)
Men	23 (88)	21 (84)
Hypercholesterolemia	7 (30)	13 (50)
Diabetes Mellitus	3 (12)	2 (8)
Hypertension	10 (39)	13 (50)
Family History of IHD	4 (17)	5 (21)
Previous MI	2 (9)	1 (4)
Previous Angina	1 (4)	1 (4)
Respiratory Disease	2 (9)	1 (4)
Smoking status		
Never	10 (38)	9 (36)
Ex-smoker	5 (19)	4 (16)
Current	11 (43)	12 (48)

	EPO (n=26)	Placebo (n=25)
Medication at presentation (%)		
Aspirin	5 (19)	5 (20)
Clopidogrel	0	0
ACE-I/ARB	4 (15)	6 (24)
Beta-blocker	1 (4)	2 (8)
CCB	3 (12)	1 (4)
Statin	4 (15)	6 (24)
Nitrate	1 (4)	0
Sulphonylurea	1 (4)	2 (8)
Metformin	2 (8)	2 (8)
PPI	1 (4)	1 (4)
Blood result at presentation		
Hb (g/dl)	14.7 (1.2)	14.2 (1.1)
Haematocrit	0.42 (0.03)	0.41 (0.03)
Platelet count (10^9/L)	256 (53)	254 (76)
Prothrombin Time (secs)	10.7 (0.7)	10.5 (0.5)
Creatinine (μmol/l)	83 (15)	79 (14)
Blood Result Day 2		
Hb (g/dl)	13.6 (1.6)	13.6 (1.6)
Haematocrit	0.40 (0.04)	0.39 (0.04)
Platelet count (10^9/L)	217 (53)	234 (83)
Creatinine (μmol/l)	83 (14)	82 (15)

Data are number (%) or mean (SD). ARB- Angiotensin receptor blocker. CCB- calcium channel blocker. PPI- proton pump inhibitor.

Table 2. Details of PPCI procedure

	EPO	Placebo
Culprit vessel		
LAD	10 (38)	13 (52)
LCx	7 (27)	4 (16)
RCA	9 (35)	8 (32)
Symptom to PPCI time, mean (mins)	224 (104)	257 (156)
Door to balloon time, mean (mins)	56 (24)	39 (16)

	EPO	Placebo
Medication during PPCI		
Asprin	26 (100)	25 (100)
Clopidogrel	26 (100)	25 (100)
Heparin	26 (100)	25 (100)
Opiate	15 (58)	13 (52)
Nitrate	13 (50)	16 (64)
Abciximab	25 (96)	23 (92)
Metoclopramide	7 (27)	10 (40)
Atropine	4 (15)	7 (28)
Mean TIMI flow before PPCI (grade)	0	0
Mean TIMI flow after PPCI (grade)	3	3
Rentrop Grade (Collateralisation)	0	0
Lesion predilated	25 (96)	23 (92)
Direct stent to lesion	1 (4)	2 (8)
Bare Metal Stent	17 (65)	16 (64)*
Drug Eluting Stent	9 (35)	10 (40)*
Mean arterial pressure, mmHg		
Pre-PCI	110 (14)	111 (22)
Post PCI	97 (16)	95 (22)
Day 2	93 (14)	95 (11)
Follow up	102 (15)	103 (17)
Median length of hospital stay, days (range)	3.0 (2 to 6)	3.5 (2 to 10)
Medication on discharge (%)		
Aspirin	25 (96)	25 (100)
Clopidogrel	26 (100)	25 (100)
ACE-I/ARB	25 (96)	25 (100)
Beta-blocker	23 (88)	20 (80)
Statin	26 (100)	25 (100)
PPI	3 (13)	1 (5)
Medication at follow-up (%)		
Aspirin	16 (89)	14 (88)
Clopidogrel	12 (67)	12 (75)
ACE-I/ARB	17 (94)	13 (81)
Beta-blocker	14 (78)	12 (75)
Statin	15 (83)	13 (81)
PPI	1 (6)	1 (6)
Median time to acute CMR scan (range)	2 (1 to 4)	2 (1 to 6)
Median time to follow-up CMR scan (range)	126 (116 to 160)	131 (106 to 163)

Data are number (%) or mean (SD). LAD- left anterior descending artery. LCx- left circumflex artery. RCA- right coronary artery. ARB- Angiotensin receptor blocker. PPI-proton pump inhibitor. *One patient received both bare metal and drug eluting stent.

Table 3. Clinical outcomes

	EPO	Placebo	Difference (95% CI)	P-value
Initial blood results, myocardial area-at-risk and CMR scan				
24 hr Troponin-T AUC (µg/l)	114.6	100.8	13.81(-29.69, 57.31)	0.526
24 hr CK-MB AUC (U/l)	4682.3	3649.1	1033.2(-562.7, 2629.1)	0.199
BARI score (%)	29	33	-3.8 (-8.6, 1.0)	0.12
APPROACH score (%)	30	31	-0.8(-6.8,5.2)	0.8
Infarct-ESA by CMR (%)	28	27	0.8(-5.4, 7.0)	0.8
Myocardial infarct mass (g)	32.8	25.4	7.46 (-2.76, 17.69)	0.148
Myocardial salvage Index	0.38	0.47	-0.09 (-0.28, 0.10)	0.333
Microvascular obstruction (%)	81.8	47.4	34.4 (6.8, 62.1)	0.020
LVEDVi (mL/m^2)	84.4	73.0	11.40 (4.05, 18.75)	0.003
LVESVi (mL/m^2)	41.3	34.6	6.77 (0.45, 13.09)	0.036
LVSVi (mL/m^2)	43.1	38.4	4.63 (-0.44, 9.70)	0.072
LVMi (g/m^2)	89.2	79.4	9.78 (0.94, 18.63)	0.031
LVEF (%)	51.3	53.2	-1.87 (-7.40, 3.66)	0.499
4 month follow-up CMR endpoints				
LVEDVi (mL/m^2)	88.1	84.4	3.74 (-10.11, 17.58)	0.586
LVESVi (mL/m^2)	42.7	36.4	6.30 (-6.60, 19.21)	0.327
LVSVi (mL/m^2)	45.4	48.0	-2.56 (-8.24, 3.11)	0.364
LVMi (g/m^2)	82.4	75.0	7.33 (-5.00, 19.56)	0.235
LVEF(%)	52.6	59.1	-6.46 (-13.94, 1.01)	0.088
Myocardial infarct mass (g)	17.7	12.4	5.31 (-0.57, 11.19)	0.075

Values are mean (SD). AUC- area-under-curve. LVEDVi- Indexed left ventricle end-diastolic volume. LVESVi- Indexed left ventricle end-systolic volume. LVSVi- Indexed left ventricle stroke volume. LVMi- Indexed left ventricle mass. LVEF- left ventricle ejection fraction.

Figure 7: Graphs to show cardiac enzyme release following PPCI.
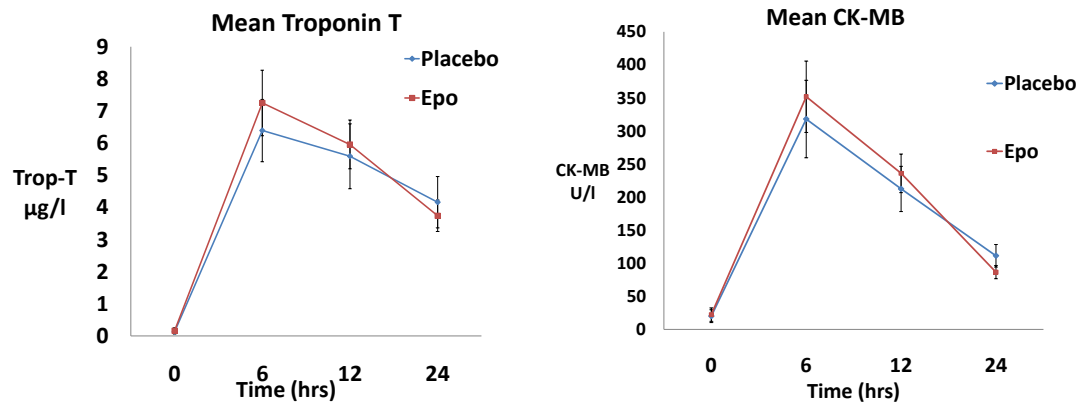
Mean serum Troponin-T (left panel) and CK-MB (right panel) concentrations over 24 hours in patients treated with PPCI. Error bars show SEM.

Figure 8 and 9: Cardiac magnetic resonance imaging results.
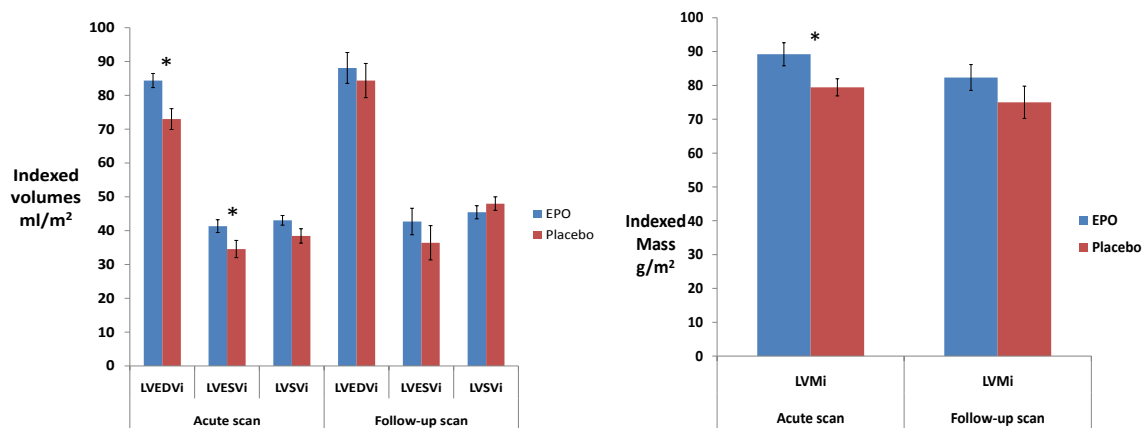
Graphs to show the indexed left ventricular end diastolic volume (LVEDVi), end systolic volume (LVESVi), stroke volume (LVESVi) and mass (LVMi) in EPO and placebo groups at the acute scan and follow up. * indicates significant result with p<0.05. Error bars are SEM.

was a trend to increased infarct size and reduced LV ejection fraction in the EPO-treated group compared to placebo.

There was good to excellent agreement between observers with the intra class correlation (ICC) ranging between 0.7 and 0.95 (table 4). Due to the trial size there was some sensitivity in the mean differences due to observations identified as outliers but there were no substantive changes in the conclusions drawn.

Table 4: Calculated inter-observer agreement for analysis of left ventricular volumes and microvascular obstruction from the magnetic resonance imaging results.

	N	Reliability (ICC)	Difference (95% Limits of agreement)
MVO (%)	41	82.6%	N/A
LVEDV (ml/m^2) (scan 1)	41	93.7%	2.70 (-16.58, 21.97)
LVESV (ml/m^2) (scan 1)	41	87.9%	1.61 (-17.16, 20.51)
Myocardial mass (g) (scan 1)	41	77.3%	-13.03 (-44.91, 18.85)
LVSV (ml/m^2) (scan 1)	41	90.5%	1.02 (-15.72, 17.76)
LVEDV (ml/m^2) (scan 2)	34	95.2%	-0.69 (-24.74, 23.36)
LVESV (ml/m^2) (scan 2)	34	95.2%	-0.17 (-21.01, 20.67)
Myocardial mass (g) (scan 2)	34	65.8%	-17.66 (-45.87, 10.54)
LVSV (ml/m^2) (scan 2)	34	85.8%	-0.52 (-19.09, 18.05)

LVEDV- Left ventricle end-diastolic volume. LVESV- Left ventricle end-systolic volume. LVSV- Left ventricle stroke volume. LVM- Left ventricle mass. MVO- microvascular obstruction.

Adverse Events

Due to the high risk nature of STEMI adverse events (see table 5) are to be expected but of course must be monitored and each reported.

Three serious adverse events were reported to the trial sponsor and ethics committee. The first was a patient (RM) who was initially deemed eligible for the study but was eventually excluded as it was discovered he had had a ventricular fibrillation cardiac arrest en route to The Heart hospital. RM was recruited to the study, allocated to the erythropoietin group and successfully completed all initial aspects. Whilst making contact to arrange the follow up scan it was discovered that he had died from a spontaneous intracranial haemorrhage. An investigation with independent review was conducted which concluded that the trial was unlikely to have played a role in this death.

The second was a patient (MA) who was recruited to the placebo arm of the study. On day 1 post-MI the patient developed an ischaemic lower limb which required vascular surgery. Post-operatively myocardial ischaemia was noted and repeat PCI planned to treat residual disease noted at the index coronary intervention. Unfortunately during this procedure the patient suffered a cardiac arrest and died. Following an independent review, the trial was found to have no role in the causation of this incident. This patient had undergone the acute CMR scan and so accounts for 1 drop out from the follow up scan.

The third patient (RN) was consented and randomised in to the EPO arm of the study. His blood pressure at the start of the cardiac catheterisation, at the same time as receiving the first dose of EPO, fulfilled criteria for cardiogenic shock. During the PPCI treatment for his inferior STEMI he sustained a cardiac arrest and subsequently died. Once again the case was reviewed with the clinical consultant and an independent reviewer who acknowledged that the onset of cardiogenic shock was prior to EPO administration and the trial was not thought

to have played any role. Due to the pre-existing cardiogenic shock and cardiac arrest, both exclusion criteria for this study, the patient was excluded. Also due to the development of cardiogenic shock and death no meaningful results were obtained from this patient.

During the initial PCI 1 patient in the EPO group and 2 in the placebo group underwent non-culprit vessel PCI. In the group eligible for analysis there was one death in the placebo group (as above). Over the follow up period 5 patients in the EPO group were admitted unplanned to hospital for investigation of chest pain versus 1 from the placebo group. 1 patient from the EPO group underwent unplanned CABG surgery and 2 patients from each group underwent PCI planned at the index admission. At follow up 1 patient was found to have a left ventricular thrombus which was brought to the attention of the clinical team. The unplanned CABG and hospital admissions in the EPO group made up a MACCE rate in the analysis group of 23% (6/26) and 8% (2/25) in the placebo group (p=NS). (Table 5).

Table 5: In-hospital adverse events rate and up to 4 months in those with a follow-up CMR scan.

	EPO	Placebo	P
Bystander PCI, n (%)	1 (4)	2 (8)	
All cause mortality, n (%)	0	1 (4)	
Cardiac Death, n (%)	**0**	**1 (4)**	
MI/Unstable Angina, n (%)	**0**	**0**	
Hospital Admission, n (%)	**5 (19)**	**1 (4)**	
Unplanned PCI or	**1 (4)**	**0**	

	EPO	Placebo	P
CABG, n (%)			
Planned PCI, n (%)	2 (8)	2 (8)	
LV Thrombus	1 (4)	0	
Total MACE, n (%)	**6 (23)**	**2 (8)**	**0.2**

Study Limitations

This clinical study was conducted in a group of patients who are seriously unwell and where there is a considerable pressure to achieve treatment within a target 'door to balloon time'. As such all patients were approached for consideration and consented provisionally pending the identification of exclusion criteria. In order to replicate the conditions of the pre-clinical studies the study entry criteria are necessarily strict. The basic science would suggest that the most benefit is achieved in this group and hence in order to demonstrate efficacy these criteria were chosen. This does have the effect however of limiting the real world applicability of the intervention. This is not necessarily a problem as some existing treatments are only efficacious in certain patient groups, but it is a matter of identifying the correct group. Subsequently although a large number of patients were consented, exclusion criteria were often identified and these patients took no further part in the study. If this study had been successful it would have potentially been necessary to look at the effect of EPO in a larger group of 'all-comers' to address efficacy in a less homogenous population. As such the data analysis is conducted on a per-protocol basis although the patients were all selected on pre-specified criteria. Clearly, whilst adequately powered, the study is of small numbers and as such the generalisability of the findings is limited. The mechanism of protection of EPO in this setting is still uncertain and we did not attempt to elucidate this any further. The numbers

of patients undergoing cardiac MRI was less than anticipated and related to a number of issues, largely patient choice but also difficulties in facilting transfer across London within 48 hours of an acute MI to the CMR unit. A CMR scanner on site (as is now the case) would have been considerably easier. Further drop out was observed between first and second scans and reflected the difficulties of ensuring 100 percent follow up in such studies.

Discussion

The major findings of this proof-of-concept randomised clinical trial are that the administration of high-dose rhEPOβ as an adjunct to PPCI had no beneficial effects in terms of myocardial infarct size, myocardial salvage index, or LV ejection fraction. In fact, the findings suggest that rhEPOβ may actually be detrimental in this setting, as it almost doubled the incidence of microvascular obstruction (MVO). Furthermore, it was associated with acute LV dilatation and an acute increase in calculated myocardial mass. On the 4 month follow-up CMR scan there were no significant differences between the treatment groups although there is a noticeable trend to increased infarct size and reduced LV ejection fraction in the EPO group. Adverse events were increased in the EPO group but not significantly so.

It is well-established that in STEMI patients successfully treated with PPCI, the presence of MVO is associated with worse clinical outcomes[342][343]. MVO can be detected in about 30-40% of PPCI patients using echocardiography[344] or CMR[292][343], even in the presence of Thrombolysis in Myocardial Infarction (TIMI) 3 coronary artery flow post-PPCI. In our study, in patients treated with rhEPOβ the incidence of MVO detected by the initial CMR scan was dramatically increased from 47% in placebo to 82%, a finding which might be expected to be associated with worse clinical outcomes. Importantly, factors which are known to impact on the development of MVO such as the presence of co-morbid conditions,

anti-platelet and anticoagulant therapy, and myocardial infarct size were not significantly different between the two treatment groups. MVO results in poor myocardial perfusion despite epicardial coronary artery revascularisation and its development has been attributed to a variety of factors including distal embolisation, endothelial dysfunction, leucocyte migration and plugging and platelet aggregation[345]. The mechanism underlying the increased incidence of MVO following PPCI in the rhEPOβ-treated group in our study is unknown but may be attributed to the increased platelet reactivity and the pro-thrombotic effects which have been described with rhEPO therapy in healthy subjects[346], in critically ill patients[347], and in patients with chronic kidney disease[254 348], cancer[349] and stroke[350]. Interestingly, high-dose rhEPO (intravenous 400 IU/kg given daily for 3 days) significantly blunted the aspirin-mediated increase in bleeding time when compared with placebo in healthy volunteers, although no effect was observed on the clopidogrel-mediated increase in bleeding time[255].

The explanation for the increased myocardial mass (calculated from the myocardial volume) observed on the initial CMR scan, in patients treated with rhEPOβ is unknown. It may have been due to enhanced myocardial oedema associated with increased myocardial injury, as suggested by the greater incidence of MVO in the rhEPOβ-treated patients. In this respect, an increased indexed myocardial mass has been noted by CMR one week following an acute myocardial infarction in a previous study[351]. As for the mechanism underlying the acute LV dilatation noted in patients treated with rhEPOβ, this may well be a compensatory response to greater myocardial injury, where an increase in preload provokes LV dilatation in an attempt to maintain the LV stroke volume.

Interestingly, the effect of high-dose rhEPO as a novel cytoprotective agent in a variety of other clinical settings of ischemia-reperfusion injury including coronary artery bypass graft (CABG) surgery[343], non-ST elevation myocardial infarction (NSTEMI)[345], and stroke[350], has also been negative. In a large phase II multicentre German study[350], the

administration of rhEPOα (40,000 IU per day for 3 days), initiated within 6 hours of stroke onset, to 223 stroke patients had no beneficial effects in terms of cerebral infarct size and functional recovery, when compared to 237 stroke patients given placebo. Importantly, rhEPOα treatment was associated with a significant increase in death rate of 16.4% compared to 9.0% in patients administered placebo at 90 days (OR, 1.98; 95% CI, 1.16 to 3.38; $P<0.01$), a significant proportion (15.2%) of which were due to thromboembolic complications[350].

In a preliminary study comprising 22 patients receiving PPCI the administration of an intravenous bolus of darbepoetin alpha (300μg equivalent to about 60,000 IU rhEPOα) did not report any safety concerns[243]. The administration of rhEPOβ (30,000 IU) prior to tenecteplase thrombolysis in STEMI patients failed to find any difference in cardiac enzyme release or any difference in major adverse cardiac events[245]. A small clinical study has reported that the administration of rhEPOα (33,000 IU daily for 3 days) to PPCI patients reduced CK-MB release but failed to limit myocardial infarct size on a CMR scan performed within 3 days of PPCI[248]. In that study, the effect of rhEPO on CMR-detected MVO was not reported.

There are several ongoing multi-centre studies investigating the effects of high-dose rhEPO in PPCI patients (including HEBE-III[352], EPAMINONDAS[353], and REVIVAL-3[354]), of which the preliminary findings from the REVIVAL-3 study were presented at last year's American College of Cardiology Scientific Sessions[354]. In that study comprising 138 PPCI patients, it was reported that treatment with rhEPOβ (33,333 IU daily for 3 days) had no difference on LV ejection fraction measured at 6 months by CMR, and that there was a trend to adverse cardiovascular outcomes (death, recurrent myocardial infarction, infarct-related artery revascularisation and stroke)[354].

In our study, the failure of rhEPOβ to reduce myocardial infarct size in PPCI patients is in conflict with the pre-clinical data reporting 50% reductions in myocardial infarct size in

rat, rabbit and dog models of ischemia-reperfusion injury, with EPO administered at time of reperfusion[337]. Interestingly, the infarct-limiting effects of EPO as reperfusion therapy in larger animal studies of ischemia-reperfusion injury such as sheep[214] and porcine[208] have been negative. Discordant findings between pre-clinical animal studies and the clinical translation of novel cardioprotective strategies has been a recurring issue, the causes of which have been highlighted in several recent articles[7 326 355]. Specifically, the pro-inflammatory and pro-thrombotic conditions associated with an acute myocardial infarction in a middle-aged patient with co-morbidities such as diabetes, dyslipidaemia and hypertension are not reproduced by experimental coronary artery occlusion in disease-free juvenile small to medium-size animal models.

In summary, we have demonstrated that the administration of high-dose rhEPOβ as an adjunct to PPCI failed to reduce myocardial infarct size or improve cardiac function. In fact, its use may actually be detrimental in that it was associated with a doubling in the incidence of MVO, acute LV dilatation and increased myocardial mass. This study highlights the importance of CMR as a technique for assessing the safety and efficacy of novel reperfusion therapies. The findings from the current study should be considered in the design of future studies investigating the use of high-dose rhEPO in patients with acute myocardial infarction.

CHAPTER 5

Targeting the reperfusion injury salvage kinase pathway in the clinical setting: Lost in translation

Introduction

This thesis has examined the use of atorvastatin and erythropoietin as pharmacological mimetics of ischaemic preconditioning in two different clinical settings of myocardial ischaemia-reperfusion injury. Neither agent was shown to have beneficial effects despite significant pre-clinical data which suggested that they would and as such this effect could be regarded as 'lost in translation'. This is not a novel issue, as since ischaemic preconditioning was first described there is yet to be a successfully translated technique which has been adopted to routine clinical practice and replicates the extraordinary protection seen in the animal setting. Coronary heart disease still represents a huge burden of morbidity and mortality (see chapter 1) and it is not doubted that further improvement in treatment is needed. Novel cardioprotective strategies are required and 'cardioprotection' has long been regarded as one method which may be successful in accomplishing this.

This chapter aims to examine the reasons behind why the two agents studied were not protective and to look at the wider issues surrounding this which have become apparent to the author during the conduct of this research degree.

Inparticular the shortcomings of the current animal experimental disease models which are used in this area of research will be examined, areas highlighted where more representative animal disease models exist, the relevance to the studies reported in this thesis will be emphasised and finally the design of clinical cardioprotection studies will be discussed in

order to increase the overall success in translating cardioprotective treatment strategies from 'bench to bedside'.

Background

Since the realisation that cardiomyocyte death was not inevitable following coronary artery occlusion the search has been on for techniques or pharmacological agents 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[356], 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'[357], and can in itself induce cardiomyocyte death (a concept termed lethal reperfusion injury and reviewed in[7]). 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 ischaemia-reperfusion injury by applying brief non-lethal episodes of ischaemia and reperfusion either to the heart itself or an organ or tissue remote from the heart. The advent of interventional strategies and pharmacological agents capable of limiting myocardial injury when applied either before or during myocardial ischaemia or at the onset of myocardial reperfusion has demonstrated the potential application for cardioprotection. The development of novel treatment strategies for protecting the myocardium against the detrimental effects of acute ischaemia-reperfusion injury and improving clinical outcomes in patients with CHD requires the use of appropriate animal disease models. Despite the ability to demonstrate this enhanced cardioprotection in a wealth of experimental animal disease models, the translation to the human clinical setting has been largely disappointing[355][358].

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[18 359], or after the onset of index myocardial ischaemia (perconditioning)[360] or even at the onset of myocardial reperfusion (postconditioning)[361 362].

Isolated *in vitro* buffer-perfused animal hearts subjected to regional or global ischaemia and reperfusion at the end of which cardiac enzyme release, infarct size or cardiac function are determined have been the mainstay for investigating potentially new treatment strategies for protecting the heart against acute ischaemia-reperfusion injury. This model is the one used in the pre-clinical study[78] upon which the clinical atorvastatin studies in this thesis are based. 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 disease models of acute myocardial infarction 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 acute myocardial infarction.

The response of the heart to acute myocardial ischaemia-reperfusion injury varies depending on the animal disease 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, 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 establish, 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 addition, in these experimental models short periods of myocardial reperfusion are used ranging from 1 to 2 hours for the *in vitro* studies and 2 hours to 72 hours for the *in vivo* infarct studies. The long-term effects 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 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 are responsible for the acute coronary occlusion. In addition, an AMI generates an acute inflammatory state not easily emulated in experimental animal disease models of infarction. Some animal disease models have attempted to mimic the scenario of an acute myocardial infarct in both rodents and larger animals. Endothelial irritants such as ferric chloride applied within a coronary artery[363 364] in order to activate the clotting cascade, thrombotic promotion with agents such as rose bengal and green light laser agitation[365] and in the carotid artery partial surgical ligation and a hypercholesterolaemic diet[366] all go some way to replicate the human scenario but these techniques are challenging to perform, are not widely used and are

unable to fully mirror the complex interplay of events occurring in acute myocardial infarction. Closed chest, catheter and balloon coronary artery occlusion has been used successfully, such as those used to investigate erythropoietin[213] [214], but requires greater operator ability and is easiest in large animals. This is not possible in all laboratories given the facilities needed 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, 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, the metabolic syndrome, atherosclerosis, and hyperlipidaemia can impact on how the heart responds to cardioprotective treatment strategies (reviewed in[367]). 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[368]. Therefore, the development of novel cardioprotective treatment strategies should involve rigorous pre-clinical testing in animal disease models which 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 normally present in a CHD patient is difficult to replicate in one animal disease model.

Translational experimental models

The testing of a cardioprotective agent in human heart tissue or controlled human in-vivo studies 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[369], 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 which 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. This is the pre-clinical model upon which our group had shown significant benefit of Epo[215] prior to commencing the clinical study undertaken in this thesis. There exists a variety of surrogate models for investigating signalling mechanisms underlying ischaemic conditioning in human volunteers and patients, and these include models of endothelial function, exercise testing, coronary angioplasty and so on (reviewed in[370]).

Designing appropriate clinical cardioprotection trials

In designing the studies which are investigated in this thesis cognisance was taken of the significant pre-clinical data in these areas and we attempted to perform proof-of-concept studies which replicated as far as possible the study conditions which had previously been successful. Whilst this is difficult and not possible to recreate conditions 100 percent successfully we feel it is important to approach studies of this sort in this manner. Too often in the past, 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 animal studies should be conducted more akin to their clinical counterparts with convincing benefit shown in multi-centre, randomised, blinded, controlled studies prior to undertaking large expensive studies in humans[355].

Furthermore, choosing the appropriate clinical setting and treatment strategy is crucial and it is imperative to take heed of the pre-clinical animal data in this respect. For example, overwhelming pre-clinical animal data suggested that cariporide (a sodium-hydrogen ion exchange inhibitor) was beneficial if administered prior to the index myocardial ischaemia as opposed to prior to myocardial reperfusion (reviewed in[371]). Therefore, it was no surprise then that the clinical studies demonstrated that this agent was cardioprotective in the setting of cardiac surgery but not in ST-elevation patients undergoing primary PCI[372]. Similarly, if the pre-clinical data suggests 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. EPO was shown to be maximally protective in animals when administered prior to reperfusion, hence our clinical study aimed to ensure that patients still had an occluded coronary artery prior to receiving their first dose of EPO.

Patient selection is crucial when designing clinical trials of cardioprotection strategies as adjuncts to myocardial reperfusion in ST-elevation MI (STEMI) patients such as the EPO study in this thesis. Furthermore, it has been suggested that 75% of STEMI patients obtain maximum benefit with prompt myocardial reperfusion alone, leaving only 25% who may actually benefit from an adjunct to myocardial reperfusion[373]. 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 does suggest that those patients most likely to benefit from a cardioprotective treatment strategy administered at the onset of myocardial reperfusion are those sustaining a large myocardial infarct (usually 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. Therefore in our Epo study we may have seen more benefit if we had restricted further the study cohort, perhaps to those patients presenting greater than 3 hours but less than approximately 9 hours in to their infarction. This of course further limits the applicability of the intervention.

If the cardioprotective strategy needs to be administered prior to or at the immediate onset of myocardial reperfusion, there is little rationale for recruiting patients which do not have a fully occluded coronary artery (TIMI <1) at presentation, although it is appreciated that the infarct-related coronary artery may spontaneously occlude or open prior to PPCI, and this can be monitored by continuous in-ambulance ECG monitoring. Other determinants of myocardial infarct size which 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 time of coronary angiography), and the presence of chest pain within 3 days (which may inadvertently precondition the patient prior to their MI).

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, more robust endpoints of clinical cardioprotection are required as surrogate markers of clinical outcome, given the large numbers of patients which are required for the definitive mortality studies. In this respect, cardiac MRI is emerging as a powerful imaging strategy capable of quantifying left ventricular dimensions and function, myocardial infarct size, the area at risk, microvascular obstruction, myocardial haemorrhage, indices which have been linked to clinical outcomes following an AMI[374]. The improved spatial resolution of MRI over and above nuclear imaging enables greater reproducibility and allows smaller sample sizes in clinical trials to be used, and a number of different measures of cardioprotection can be made within one imaging modality, increasingly making it the modality of choice in assessing surrogate outcomes of cardioprotection. Without cardiac MRI it is unlikely that in our Epo study we would have been able to document the increased incidence of microvascular obstruction, other techniques are not particularly sensitive in this regard.

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 (5 to 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%- a phenomenon termed ischaemic postconditioning[29 375 376]. In the clinical setting this has been achieved by 3-6 cycles of inflation and deflation of a coronary angioplasty balloon following deployment of the stent within the infarct-related coronary artery, a protocol which has been reported to reduce myocardial infarct size at 6 months and preserve LV ejection fraction at one year[33].

Similarly, pre-clinical animal infarct data has established that pharmacologically inhibiting the mitochondrial permeability transition pore (mPTP), a non-selective channel of the inner mitochondrial membrane which mediates cell death by opening at the onset of myocardial reperfusion, reduces myocardial infarct size by 30-40%[377] [378] and preserves LV ejection fraction, and improves survival[379]. A recent proof-of-concept clinical study has demonstrated that as an adjunct to PPCI, cyclosporine-A is capable of reducing myocardial infarct size in STEMI patients[380]. Clearly, for both these treatment interventions, large multicentre studies are required to determine their effect on clinical outcomes. One could hypothesise that physical methods of preconditioning are able to act on several end-effectors of preconditioning and as such have a 'stronger' protective stimulus; as opposed to pharmacological agents such as atorvastatin and erythropoietin which would appear to target a limited range of cellular protective pathways which perhaps have a limited effect when put in the complex clinical context.

The effect of acute high dose atorvastatin on myocardial injury during coronary artery bypass graft surgery

The above study described in Chapter 3 of this thesis unfortunately failed to translate the expected benefit seen in pre-clinical animal and human tissue experiments in to the human clinical setting. As discussed in chapter 3 there are a number of possible reasons as to why the study results did not show cardioprotection however the basis for the study was sound given the animal evidence available. However there are perhaps one or two key areas that bear highlighting. The first is that the basis for the study was the ability to 'recapture' the waning cardioprotective effect of 'statins' based on Mensah's report[78]. The ability to add an acute 'statin' dose and recapture cardioprotection has not been validated in other animal studies. However the waning of cardioprotection with chronic statin use has been replicated[87]

and then in the human setting of percutaneous coronary intervention an acute dose of atorvastatin demonstrated benefit[123], although the benefit was largely driven by patients who had recently experienced an acute coronary syndrome. So it would seem that the principles of the study hold out- the cardioprotection of 'statins' wanes, and the ability to 'recapture' the cardioprotection is possible- but unfortunately there is a lack of a directly comparable animal study. If it were undertaken this study it would ideally use a model of bypass surgery in an elderly large mammal with a metabolic syndrome phenotype and clearly this would provide significant insights. However as discussed above the expertise and costs involved in complex large animal models often make them prohibitive.

The second issue to highlight is that the ARMYDA Recapture study[123] which demonstrated a benefit from acute statin use on a background of chronic use in PCI did so largely in those patients who had recently presented with an acute coronary syndrome. Clearly there are likely to be significant differences in elective versus urgent/emergency patients in inflammatory state, atherosclerotic plaque stability, platelet reactivity etc and so the elective group will be less likely to sustain myocardial injury making demonstrating protection difficult. The study reported in chapter 3 recruited only elective patients in order to maintain homogeneity and to prevent the wide differences in ACS presentation making interpretation of data difficult. This may well have been a significant limitation of the study and in-retrospect recruiting all patients, elective and urgent, but then adjusting for confounding factors may well have provided a wider view of the effects of acute atorvastatin.

The third factor is the remaining uncertainty of the whole mechanism of ischaemic preconditioning and pharmacological mimetics of this. Although thought to be fairly well elucidated (discussed in chapter 1) the cell survival pathways attributed to IPC are multitude and it is not clear which may play a dominant role in which setting. The RISK pathway, thought to be central to the protective effect of 'statins' has a great deal of supporting

evidence and yet this is not always consistent[381 382] and so questions still exist as to the exact mechanism. There have been an increasing number of small clinical trials that have demonstrated the cardioprotective benefit of physical interventions such as remote ischaemic preconditioning[322 329 383 384] and post-conditioning[30 33]. It is possible that at present pharmacological agents (possibly cyclosporine excepted[380]) are not able to activate the breadth of survival pathways, that perhaps a more intense physical stimuli is able to do, in order to demonstrate significant protection.

Effect of erythropoietin as an adjunct to primary percutaneous coronary intervention

The above study (chapter 4) also failed to demonstrate cardioprotective benefit and surprisingly erythropoietin in the setting of acute STEMI was associated with detrimental effects, an increase in the incidence of MVO and acute LV dilatation in the treated group. Once again there is a wealth of animal data supporting beneficial effects of Epo in the setting of reperfusion injury (see chapters 1 and 3) and there are pilot studies in brain[240] and cardiac injury[243] which have demonstrated safety and suggested the potential for benefit however, once again, a pharmacological preconditioning mimetic has failed to translate to the human clinical setting. Importantly a number of other recently published studies also appear to be demonstrating similar results, with a large study in stroke showing a worse outcome in the Epo treated group[350], and in a recently reported study in acute STEMI (REVIVAL-3[354]). Of note it may be significant that the large mammal experiments performed with Epo failed to show any significant myocardial infarct size reduction[213 214]. It remains possible that any beneficial effect of Epo is small in the majority of cases where reperfusion alone provides maximal benefit. As discussed above, treating those patients with large AAR, beyond 3 hours but within 6-9 hours of symptom onset may demonstrate that further protection is possible.

However if adverse effects of Epo use in this setting remain a concern then any benefit may be outweighed by the detrimental effects and ethically further study would be difficult until the details were clearer.

Furthermore there has been a recent paper challenging the pleiotropic abilities of Epo and the presence of biologically active Epo R in tissues not involved in erythropoeisis[385] however this paper has been criticised by a large body of researchers for overstating its conclusions[386] and not providing enough evidence for which to discount the work of a large body of other investigators. Thus, the debate as to the exact abilities of Epo continues.

Conclusion

Both atorvastatin and erythropoietin have shown significant potential for cardioprotection in the pre-clinical setting and it was hoped that considerable benefit from their acute use in preventing reperfusion injury would be realised. This thesis has examined both these agents in two different clinical settings of ischaemia-reperfusion injury and as has been discussed neither was demonstrated to show cardioprotection. This is unfortunately characteristic of this field of research, where huge strides have been made in clarifying mechanistic pathways but no real translation has occurred to routine clinical use. Therefore 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 (1) the design of suitable pre-clinical animal disease models more accurately reflect the clinical setting under scrutiny and (2) that the design of clinical cardioprotection trials take 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 endpoints (such as cardiac MRI) before multi-centre clinical evaluation of clinical outcomes.

APPENDIX 1

Research in the setting of acute myocardial infarction

The ongoing mortality and morbidity arising from acute MI necessitates the innovation and testing of new techniques, devices or pharmacological agents which will improve the current standard of care. Although animal and human tissue experiments in the laboratory are able to provide detailed mechanistic insight and suggest that a new innovation will be beneficial there will always come a time where experiments in humans must be conducted. The ethical conduct of research is not always straightforward and throughout history the trial subject has not always been safeguarded. Currently standards of trial conduct are carefully regulated to ensure that the participants are protected as much as possible. The setting of acute myocardial infarction is however an interesting and sometimes difficult area in which to conduct research because time to treatment is of the essence. In the following section the difficulties involved in gaining informed patient consent and methods of how trials may be conducted in this setting are briefly discussed.

Conduct of research

Physicians have always been pioneers and have led attempts to improve the possibility of a cure for their patient. However, not all physicians have been altruistic in their ambitions and sometimes (whether deliberately or not) the interests of the individual have been overridden in the quest for greater knowledge[387]. Following research atrocities which occurred during the second world war, the Nuremberg code was drawn up to guide human research and attempt to prevent similar events, although was never enshrined as law in most countries. The Declaration of Helsinki was drawn up and accepted in 1964 by the World Medical

Association[388], and exists today in updated form[389], as a guide to the ethical conduct of human research trials in the modern environment. In the United Kingdom the conduct of research is very tightly regulated in legislative law and overseen by the National Research Ethics Service (NRES) and the Medicines and Healthcare products Regulatory Agency (MHRA). Depending on the type of investigation, whether a clinical trial of investigational medicinal product (CTIMP) or not (non-CTIMP) decides as to whether the study is regulated by the MHRA or NRES respectively. The studies conducted within this thesis were deemed of a mechanistic nature by the MHRA and therefore did not need their approval and oversight was provided by the local research and development (R&D) department and local ethics committee.

Consent

One of the basic principles protecting participants in research is the need to gain informed consent (usually written) prior to their inclusion in the study. In a planned situation such as elective CABG surgery (see chapter 3) this is relatively straightforward and enables the patient to fully consider whether or not they wish to take part. In the setting of acute myocardial infarction the situation is more difficult. The time to reperfusion therapy is crucial and research studies cannot be allowed to delay treatments that are known to be beneficial. Often the patient is alert and able to comprehend their situation and take in information given to them, however the spectrum is wide and many patients may be in too much discomfort, have received opiate medication or may even have suffered cardiac arrest rendering them lacking the capacity to make an informed decision about their immediate treatment let alone taking part in research. The study detailed in this thesis investigating the cardioprotective benefits of Epo in STEMI (chapter 4) was conducted along the traditional lines of gaining written consent during the acute situation for the patients' inclusion in the study. This method

was relatively successful however it is debatable exactly how much information each patient was able to retain in the acute situation and the investigators took great care to fully explain and in effect re-consent the patient after they had been stabilised. Along similar lines treatment by PPCI for STEMI currently requires written consent but again this would appear to be very difficult to achieve properly in the acute situation and it has been suggested that in fact verbal consent, adjusted to the situation would be less stressful for the patient and would expedite treatment at this critical time[390]. It is worth remembering that it is the consent process which is important and not just the patients' signature on a form.

Patients who lack capacity

Patients suffering from a STEMI may temporarily lack capacity to make informed decisions regarding their treatment or their participation in clinical research. Recent legislation for non-CTIMPs has helped researchers clarify this difficult area. The Mental Capacity Act 2005[391] has acknowledged that research may sometimes need to be conducted in patients lacking capacity and as such, subject to safeguards, has made provision for this. Sections 30 to 34 of the Mental Capacity Act 2005 deal very well with issues of diminished capacity and enrolment in to clinical studies. Far from being too restrictive to those wishing to study groups of patients with diminished capacity the Act is supportive as long as the patient group being studied must necessarily be recruited at a time when capacity is diminished and time is of the essence e.g. studies in acute myocardial infarction. Section 31, paragraph 5a, highlights that the research study must have, '...the potential to benefit the patient (P) without imposing on P a burden that is disproportionate to the potential benefit to P...'

For researchers it is important to note that the act does not apply necessarily to trials which fall under the Medicines for Human Use Regulations 2004, as separate legislation and guidance exists.

Researchers must work closely with their local ethics committee to ensure that the consent process is appropriate to the situation and that the stipulations of the Act are followed but it is also important to realise that research in patient groups with diminished capacity is essential in some circumstances and that the Act clearly allows for this[392].

In the setting of acute MI following careful ethical committee approval it may be possible to include patients in research studies by adjusting the consent process to their capacity at the time and then allowing further informed consent at a later time point. This would have the additional benefit of not selecting out from studies the most ill patients who are unable to consent but who may have much to gain from further innovation[393].

This is the approach that was formulated and agreed with the local ethics committee for the running of the PREP-MI study (Pre-hospital REmote Ischaemic Postconditioning in Myocardial Infarction) which I have subsequently commenced and although the study is in the early stages this method of consent appears successful.

APPENDIX 2

Effect of erythropoietin as an adjunct to primary percutaneous coronary intervention: a randomised controlled clinical trial.

In order to further examine the effect of erythropoietin in STEMI a further exploratory post-hoc analysis of group characteristics and outcomes has been performed by culprit vessel. As a sub-group analysis patient numbers are small and so the ability to form further conclusions is limited.

Table 1: Patient characteristics by culprit artery

	Epo (n=26)			Placebo (n=25)			P value
	LAD	RCA	LCx	LAD	RCA	LCx	
N	10	9	7	13	8	4	
Age, mean yrs (SD)							>0.05
Men (%)	80	89	100	92	75	75	>0.05
Hypercholesterolaemia (%)	40	11	29	46	63	50	>0.05
Diabetes Mellitus (%)	20	11	0	15	0	0	>0.05
Hypertension (%)	70	11	29	54	50	50	>0.05
Family History of IHD (%)	0	22	29	15	38	0	>0.05
Previous MI (%)	10	0	14	0	13	0	>0.05
Previous Angina (%)	0	11	0	0	13	0	>0.05
Respiratory Disease (%)	0	22	0	0	13	0	>0.05
Smoking status (%)							>0.05
Never	60	33	29	23	49	50	
Ex	20	22	14	23	13	0	
Current	20	55	57	54	38	50	
Medication at presentation (%)							>0.05
Aspirin	20	22	14	15	25	25	
Clopidogrel	0	0	0	0	0	0	
ACEI/ARB	30	11	0	15	38	25	
Beta-blocker	0	0	14	8	13	0	
CCB	10	11	14	8	0	0	
Statin	30	11	14	23	13	50	
Nitrate	0	11	0	0	0	0	
Sulphonylurea	10	22	0	0	0	0	
Metformin	20	22	0	0	0	0	
PPI	10	0	0	0	13	0	

Table 2: Interventional Details

	EPO (n=26)			Placebo (n=25)		
	LAD	RCA	LCx	LAD	RCA	LCx
Culprit vessel, N (%)	10 (38)	9 (35)	7 (27)	13 (52)	8 (32)	4 (16)
Symptom to reperfusion time, mins (SD)	229 (120)	208 (96)	237 (102)	260 (165)	289 (175)	182 (54)
Medication peri-PCI (%)						
Asprin	100	100	100	100	100	100
Clopidogrel	100	100	100	100	100	100
Heparin	100	100	100	100	100	100
Opiate	50	66	57	69	38	25
Nitrate	40	33	86	77	63	25
Abciximab	90	100	100	92	100	75
Metoclopramide	20	33	29	54	25	25
Atropine	0	33	14	8	63	25
Mean TIMI flow before PCI, (grade)	0	0	0	0	0	0
Mean TIMI flow after PCI (grade)	2.92	2.9	3	2.9	3	3
Predilated (%)	90	100	100	92	88	100
Direct stent (%)	10	0	0	8	12	0
Bare Metal Stent (%)	50	100	43	54*	75	75
Drug Eluting Stent (%)	50	0	57	54*	25	25
Mean arterial pressure, (mmHg)						
Pre-PCI	111	108	113	109	112	115
Post PCI	99	95	98	94	88	111
Day2	93	89	99	96	94	97
Follow up	104	106	97	94	106	133
Mean length of hospital stay, days (SD)	4.1 (1.5)	3 (0.9)	3.6 (1.4)	3.7 (2.1)	4.6 (1.6)	5 (2.1)

*One patient received both bare metal and drug eluting stents.

Table 3: Cardiac enzyme release by culprit vessel

Troponin T (μg/l ± SD)

	Vessel	0 hrs	6 hrs	12 hrs	24 hrs	AUC
EPO	LAD	0.04 ± 0.06	9.69 ± 6.12	7.30 ± 4.17	4.09 ± 2.82	131±94
	RCA	0.15 ± 0.34	6.14 ± 4.74	5.54 ± 4.14	3.51 ± 2.34	108±80
	LCx	0.35 ± 0.78	5.57 ± 3.16	4.77 ± 2.68	3.67 ± 2.29	99±55
Placebo	LAD	0.17 ± 0.44	8.79 ± 5.19	6.3 ± 5.96	4.44 ± 3.53	110±93
	RCA	0.12 ± 0.19	3.44 ± 1.84	4.27 ± 1.5	2.64 ± 0.96	75±76
	LCx	0.04 ± 0.04	4.67 ± 2.25	6.64 ± 5.94	6.41 ± 7.07	119±84
P Value		NS	NS	NS	NS	NS

Table 4: Imaging DATA by Culprit Artery

	EPO			Placebo			P
	LAD	RCA	LCx	LAD	RCA	LCx	
Patients with acute MRI %	38	23	23	40	32	8	>0.05
Patients with acute and follow up MRI %	27	23	19	32	28	4	>0.05
Days post MI of first MRI, mean (SD)	2.1	1.5	2	2.1	2.1	5.5	>0.05
Days between MRI scans, mean (SD)	130 (18)	133 (16)	123 (5)	136 (14)	130 (15)	152 (-)	>0.05

Table 5: Area At Risk by culprit vessel

	EPO			Placebo			P
	LAD	RCA	LCx	LAD	RCA	LCx	
BARI (%)	33	25	27	39	27	23	NS
APPROACH (%)	38	24	25	38	25	17	NS
MRI ESA (%)	31	29	22	32	22	22	NS

Table 6: Acute Scan CMR results by culprit vessel

	Trial intervention	Vessel	Mean	Std. Deviation
MRI ESA Mass (g)	EPO	LAD	49	17
		RCA	43	9
		LCx	39	18
	Control	LAD	48	24
		RCA	29	6
		LCx	30	2
EDV (ml)	EPO	LAD	**162**	**24**
		RCA	**163**	**19**
		LCx	**170**	**18**
	Control	LAD	147	23
		RCA	138	30
		LCx	117	48
ESV (ml)	EPO	LAD	**80**	**18**
		RCA	**81**	**16**
		LCx	**79**	**18**
	Control	LAD	74	18
		RCA	56	12
		LCx	48	24
SV (ml)	EPO	LAD	82	19
		RCA	81	6
		LCx	91	15
	Control	LAD	73	13
		RCA	82	25
		LCx	69	24
EF (%)	EPO	LAD	51	8
		RCA	50	7
		LCx	54	9
	Control	LAD	50	8
		RCA	59	8
		LCx	60	4

	Trial intervention	Vessel	Mean	Std. Deviation
CO (L/min)	EPO	LAD	6.2	1.4
		RCA	6.1	0.68
		LCx	5.8	1.4
	Control	LAD	5.4	1.3
		RCA	6.0	1.5
		LCx	4.4	1.7
Myocardial Mass (g)	EPO	LAD	157	34
		RCA	149	25
		LCx	167	40
	Control	LAD	146	29
		RCA	139	26
		LCx	136	14
Infarct Volume (ml)	EPO	LAD	33	16
		RCA	31	10
		LCx	29	18
	Control	LAD	29	21
		RCA	20	5
		LCx	16	12
Infarct Mass (g)	EPO	LAD	35	17
		RCA	32	11
		LCx	30	19
	Control	LAD	30	22
		RCA	22	6
		LCx	17	12
Infarct Size (% of LV myocardium)	EPO	LAD	22	10
		RCA	22	5
		LCx	17	8
	Control	LAD	20	12
		RCA	16	5
		LCx	12	8
MVO (%)	EPO	LAD	**70**	**-**
		RCA	**100**	**-**
		LCx	**83**	**-**
	Control	LAD	56	-
		RCA	57	-
		LCx	0	-
LVEDVi (ml/m^2)	EPO	LAD	**83**	**12**
		RCA	**83**	**9**
		LCx	**87**	**8**
	Control	LAD	76	11
		RCA	72	15
		LCx	60	19
LVESVi (ml/m^2)	EPO	LAD	**42**	**10**
		RCA	**41**	**9**
		LCx	**41**	**10**

	Trial intervention	Vessel	Mean	Std. Deviation
LVSVi (ml/m²)	Control	LAD	38	10
		RCA	29	6
		LCx	24	10
	EPO	LAD	42	8
		RCA	42	5
		LCx	46	5
	Control	LAD	38	6
		RCA	43	12
		LCx	36	9
CI (l/min/m²)	EPO	LAD	3.2	0.5
		RCA	3.1	0.5
		LCx	2.9	0.4
	Control	LAD	2.8	0.6
		RCA	3.1	0.7
		LCx	2.2	0.7
LVMi (g/m²)	EPO	LAD	**80**	**13**
		RCA	**76**	**8**
		LCx	**85**	**13**
	Control	LAD	75	13
		RCA	72	11
		LCx	71	0.1

LVEDV(i)- Left ventricle end-diastolic volume (indexed). LVESV(i)- Left ventricle end-systolic volume (indexed). LVSVi- Left ventricle stroke volume (indexed). LVMi- Left ventricle mass (indexed). LVEF- left ventricular ejection fraction. CI- cardiac index. MVO- microvascular obstruction. LAD- left anterior descending artery, RCA- right coronary artery, LCx- left circumflex artery.

Table 7: Follow-up Scan CMR results by culprit vessel

	Trial intervention	Vessel	Mean	Std. Deviation
EDV (ml)	Control	LAD	165	50
		RCA	152	25
		LCx	212	-
	EPO	LAD	165	14
		RCA	180	52
		LCx	162	49
ESV (ml)	Control	LAD	72	47
		RCA	59	12
		LCx	144	-
	EPO	LAD	82	13
		RCA	86	44

	Trial intervention	Vessel	Mean	Std. Deviation
		LCx	68	26
SV (ml)	Control	LAD	93	11
		RCA	93	21
		LCx	69	-
	EPO	LAD	83	12
		RCA	95	15
		LCx	94	28
EF (%)	Control	LAD	59	14
		RCA	61	7
		LCx	32	-
	EPO	LAD	50	6
		RCA	55	10
		LCx	58	5
CO (L/min)	Control	LAD	5.9	0.7
		RCA	5.8	1.4
		LCx	3.8	-
	EPO	LAD	5.3	0.6
		RCA	6.5	0.7
		LCx	5	1.4
Myocardial Mass (g)	Control	LAD	**119**	**29**
		RCA	**119**	**30**
		LCx	**141**	**-**
	EPO	LAD	134	24
		RCA	150	38
		LCx	131	32
Infarct Volume (ml)	Control	LAD	11.8	10.8
		RCA	10.4	4
		LCx	7	-
	EPO	LAD	19.7	8.1
		RCA	17.2	5.5
		LCx	9.4	6.6
Infarct Mass (g)	Control	LAD	12.4	11.3
		RCA	10.9	4.2
		LCx	7.3	-
	EPO	LAD	20.7	8.5
		RCA	18	5.8
		LCx	9.9	7
Infarct (% of LV myocardium)	Control	LAD	10.1	9.2
		RCA	10.2	6.2
		LCx	5.5	-
	EPO	LAD	15.6	6.1
		RCA	12	2
		LCx	6.9	3.5
MVO	Control	LAD	0	-
		RCA	0	-

	Trial intervention	Vessel	Mean	Std. Deviation
		LCx	0	-
	EPO	LAD	0	-
		RCA	0	-
		LCx	25	-
LVEDVi (ml/m²)	Control	LAD	82	23
		RCA	80	12
		LCx	110	-
	EPO	LAD	85	8
		RCA	93	30
		LCx	82	14
LVESVi (ml/m²)	Control	LAD	36	23
		RCA	31	7
		LCx	75	-
	EPO	LAD	42	7
		RCA	44	25
		LCx	34	9
LVSVi (ml/m²)	Control	LAD	46	4
		RCA	49	10
		LCx	36	-
	EPO	LAD	43	7
		RCA	49	9
		LCx	48	9
CI (l/min/m²)	Control	LAD	2.9	0.4
		RCA	3.0	0.7
		LCx	2.0	-
	EPO	LAD	2.7	0.2
		RCA	3.4	0.4
		LCx	2.6	0.7
LVMi (g/m²)	Control	LAD	**59**	**12**
		RCA	**63**	**15**
		LCx	**73**	**-**
	EPO	LAD	69	12
		RCA	78	22
		LCx	67	8

LVEDV(i)- Left ventricle end-diastolic volume (indexed). LVESV(i)- Left ventricle end-systolic volume (indexed). LVSVi- Left ventricle stroke volume (indexed). LVMi- Left ventricle mass (indexed). LVEF- left ventricular ejection fraction. CI- cardiac index. MVO-microvascular obstruction. LAD- left anterior descending artery, RCA- right coronary artery, LCx- left circumflex

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