Cardioprotective growth factors

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1. Introduction

Elucidating the mechanistic pathways underlying normal cardiac development, growth, and differentiation has resulted in the identification of a variety of growth factors, each capable of exerting a diverse array of effects, many of which appear unrelated to their originally proposed function within the cardiovascular system. Several of these growth factors are released by cardiomyocytes during myocardial ischaemia, suggesting that they may play a role in cardiac repair, myocardial angiogenesis, and myocardial infarct repair. Whether their release during myocardial ischaemia confers endogenous cardioprotection has not been fully resolved and is the subject of ongoing investigation.

Interestingly, the exogenous administration of several of these growth factors has been reported to protect the myocardium from the detrimental effects of acute ischaemia–reperfusion injury. This protective effect is due to the recruitment of specific intracellular signal transduction pathways linked to cardioprotection, which include the signalling components of the reperfusion injury salvage kinase (RISK) pathway, a group of pro-survival kinases that include PI3K-Akt and MEK1/2-Erk1/2, which confer powerful cardioprotection when specifically activated at the time of myocardial reperfusion.1–3 However, it must be appreciated that the signal transduction pathways underlying the effects of these cardioprotective growth factors are complex, and a comprehensive review of all the signalling pathways involved is beyond the scope of this review article. Therefore, in this article, we will focus on those growth factors which were originally identified as having the potential to exert cardioprotection through the RISK pathway at the onset of myocardial reperfusion, a clinically relevant time point for acute myocardial infarction patients undergoing reperfusion using either fibrinolytic therapy or primary percutaneous coronary intervention. These include the angiogenic growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), the IL-6 family cytokine member, cardiotrophin-1 (CT-1), insulin and insulin-like growth factor (IGF), transforming growth factor-β (TGF-β), and the peptide hormone, urocor- tin, which through a variety of receptor subtypes share the ability to confer cardioprotection through the activation of the RISK pathway. These growth factors have specific cardiovascular effects separate from their cardioprotective properties which may in some cases limit their eventual clinical application in patients with coronary heart disease. The reader is directed to several other reviews for comprehensive accounts on the cardioprotective effects of other peptide hormones (kinins, natriuretic peptides, and adrenomedullin),4 erythropoietin,5 and the adipocytokines.6

2. The reperfusion injury salvage kinase pathway

The initial concept that there existed pro-survival protein kinase signalling cascades capable of mediating cardioprotection at the time of myocardial reperfusion was originally proposed by our laboratory in 1999.7 The idea was formulated following the general appreciation that apoptotic cell death was essentially a reperfusion phenomenon and...
that the activation of particular anti-apoptotic protein kinases such as PI3K-Akt and MEK1/2-Erk1/2, specifically at the clinically relevant time of myocardial reperfusion, had the ability to attenuate lethal myocardial reperfusion injury and limit myocardial infarct size. The original studies which had been designed to investigate this concept had examined the effect of growth factors (including those reviewed in this article) that were known to activate PI3K-Akt and MEK1/2-Erk1/2 in order to determine whether they were capable of mediating cardioprotection when administered at the onset of myocardial reperfusion. Subsequently, a diverse variety of agents including natriuretic peptides, 'statins', adipocytokines, adenosine, bradykinin, opioids have been reported to target lethal myocardial reperfusion injury and limit myocardial infarct size when given at the time of myocardial reperfusion (reviewed in references 1 and 3).

It is important to appreciate that the use of growth factors as an acute cardioprotective strategy requires the transient short-lived activation of the PI3K-Akt and MEK1/2-Erk1/2 components of the RISK pathway as opposed to their chronic activation which can result in the loss of their cardioprotective effect and lead to unwanted cardiovascular effects such as cardiac hypertrophy. Furthermore,
the concept of the RISK pathway continues to evolve as the number of described cardioprotective agents increases and the list of other signalling pathways such as JAK-STAT, PKG, PKC, sphingosine kinase, and so on, which are capable of protecting at the time of myocardial reperfusion, is expanded.

3. Cardioprotective growth factors: protein tyrosine kinase receptors

In this section, cardioprotective growth factors which bind to protein tyrosine kinase receptors are reviewed and these include FGF, VEGF, insulin, and IGF. Growth factor binding to its specific protein tyrosine kinase receptor in the heart has the ability to activate both the PI3K-Akt and MEK1/2-Erk1/2 signal transduction cascades of the cardioprotective RISK pathway (Figure 1). The activation of the protein tyrosine kinase receptor by its specific growth factor results in the autophosphorylation of tyrosine residues, leading to the recruitment to the membrane of PI3K (class Iα), which is activated by directly binding to phosphotyrosine residues of the growth factor receptor. PI3K then generates the lipid product phosphatidylinositol-3,4,5-trisphosphate, which in turn recruits to the membrane signalling proteins with pleckstrin homology domains, including the protein serine–threonine kinases, Akt. In addition, growth factor binding to the receptor tyrosine kinases results in the activation of Raf, leading to the recruitment of Raf to the membrane and the subsequent activation of the MEK1/2-Erk1/2 kinase cascade.

3.1 Fibroblast growth factor

In 1974, Gospodarowicz isolated from bovine pituitary extract a polypeptide which was capable of stimulating fibroblast proliferation, which was termed fibroblast growth factor. Subsequent investigation has identified 23 members of the FGF family, and these regulate a diverse variety of effects including embryogenesis, angiogenesis, growth, and cell survival, although many of the experimental studies have focused on the actions of FGF-1 (formerly termed acidic or aFGF) and FGF-2 (formerly termed basic or bFGF). Both FGF-1 and FGF-2 are secreted by cardiomyocytes in response to myocardial ischaemia and they bind to a specific plasma membrane tyrosine kinase FGF1 receptor, which is known to be present on cardiomyocytes. There is an extensive literature on the myocardial angiogenic/arteriogenic properties of FGF, and its therapeutic potential for treating patients with chronic ischaemic heart disease, a topic which is comprehensively reviewed elsewhere.

In 1992, Yanagisawa-Miwa et al. were the first to demonstrate an acute cardioprotective effect in canine hearts using an intracoronary administration of FGF-2. In this particular study, the observed infarct-limiting effect of FGF-2 was attributed to its angiogenic properties. A number of subsequent experimental studies have since demonstrated an acute cardioprotective effect with FGF which is independent of its angiogenic/arteriogenic actions (Table 1). An initial experimental study by Padua et al. demonstrated infarct limitation in the isolated rat heart pre-treated with FGF-2, and a subsequent study using the in situ canine hearts demonstrated infarct size reduction with intracoronary FGF-2 in the absence of any effects on either coronary collaterals or myocardial angiogenesis. Further experimental studies have used shortened reperfusion times in order to investigate the intracellular mechanistic pathways underlying the acute cardioprotection elicited by FGF and are summarized in Table 1.

3.1.1 Signal transduction pathways underlying fibroblast growth factor cardioprotection

FGF-1 and FGF-2 exert their cardioprotective effects by binding to specific plasma membrane tyrosine kinase FGF receptor (FGFR1 in the heart), which result in the recruitment of a number of different signal transduction pathways (Figure 1).

Hun et al. were the first to demonstrate that the infarct-limiting effects of FGF-2 pre-treatment could be abolished by non-specific pharmacological antagonists of growth factors and tyrosine kinase, suggesting that the cardioprotective effect of FGF-2 may be receptor-mediated. This finding was later confirmed in an experimental study by Jiang et al. in which it was found that a mutant form of FGF-2 with reduced affinity for the FGFR-1 tyrosine kinase receptor failed to limit myocardial infarct size in the intact canine heart. This mutant form of FGF-2 was still able to bind to heparin-binding receptors and accumulate in the extracellular matrix, suggesting that this was not required for the acute cardioprotective effect.

Subsequent experimental studies have gone on to implicate other familiar mediators of cardioprotection including PKC-α, PKC-β, Erk1/2, JNK MAPK, and PKC-β. Crucially, the treatment of isolated adult rat cardiomyocytes with FGF-2 mediated cardioprotection through the activation of PKC-α, suggesting a direct protective effect of this growth factor on the cardiomyocyte. Importantly, from a clinical perspective, it has also been demonstrated by Cuevas et al. that FGF-1 was able to reduce myocardial infarct size in the in vivo rat heart when administered at the onset of myocardial reperfusion. This cardioprotective effect elicited at the onset of myocardial reperfusion was confirmed using FGF-2 in a subsequent experimental study and was linked to the activation of PKC isoforms α, ε, δ, and ζ. Importantly, the cardioprotective effect elicited by FGF-2 was demonstrated to be independent of its mitogenic effects, as a non-mitogenic mutant form of FGF-2 was capable of reducing myocardial infarct size in the perfused rat heart when administered at the onset of myocardial reperfusion, providing reassuring evidence that an acute cardioprotective strategy using FGF-2 may offer beneficial effects in terms of infarct limitation without a mitogenic effect.

The role of endogenous FGF in the context of cardioprotection using gain and loss of function approaches has also been studied. Sheikh et al. demonstrated that the transgenic overexpression of FGF-2 in the heart conferred resistance against acute myocardial ischaemia–reperfusion injury and this cardioprotective effect was associated with the upregulation of p38 MAPK (mitogen-activated protein kinase), JNK MAPK, and PKC (α and ε isoforms), although the contribution of these protein kinases to FGF cardioprotection was not investigated. In contrast, it has been reported that mice overexpressing FGF-2 are more susceptible to isoproterenol-induced myocardial injury, the mechanism of which has been attributed to pro-inflammatory
<table>
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<td>Isolated working murine heart</td>
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<tr>
<td>House et al. (2005)</td>
<td>Isolated working murine heart</td>
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MI, myocardial infarct; CPK, creatine phosphokinase.
myocardial injury mediated by T cells. The role of FGF-2 as an endogenous cardioprotective growth factor was revealed in a subsequent experimental study by Hause et al. in which it was reported that transgenic mice lacking cardiac-specific FGF-2 were more susceptible to acute myocardial ischaemia–reperfusion injury with respect to myocardial function, although there was no difference in myocardial infarct size. However, transgenic mice overexpressing cardiac-restricted FGF-2 that were subjected to myocardial infarction developed smaller infarctions and had improved function. Finally, the absence of FGF-2 had a negative impact on myocardial infarct repair and LV remodelling. It is interesting to note that the PI3K-Akt component of the RISK pathway has not been linked to the FGF cardioprotection even though this pathway is known to be activated following FGF binding to its tyrosine kinase receptor on the cardiomyocyte.

3.1.2 Clinical application of fibroblast growth factor
The clinical application of FGF thus far has been restricted to its potent ability to stimulate myocardial angiogenesis and arteriogenesis. However, subsequent clinical studies (AGENT1-4) which have examined the use of chronic treatment using adenoviral transfection with FGF-4 in patients with maximally treated chronic ischaemic heart disease reported no beneficial effects (reviewed in reference 29). Whether the acute treatment with exogenous FGF has the ability to reduce myocardial infarct size when administered as adjunctive therapy to myocardial reperfusion in patients presenting with an acute myocardial infarction remains to be determined. Previous experience in patients with stable coronary artery disease using intracoronary recombinant FGF-2 at doses <30 μg/kg had no significant beneficial effects in terms of reducing angina symptoms, and a significant number of patients experienced hypotension with the single bolus infusion, which would limit its use in patients presenting with an acute myocardial infarction unless a safe efficacious non-hypotensive dose could be found.

3.2 Vascular endothelial growth factor
VEGF, a 45 kDa polypeptide, is a major regulator of angiogenesis in the heart, which, like FGF, has been examined in clinical trials as a therapeutic angiogenic strategy in chronic ischaemic heart disease. In contrast, given that VEGF promotes angiogenesis in tumour growth, VEGF antagonists are also being developed as novel anti-cancer therapy. VEGF is generated in response to myocardial ischaemia and binds to two high-affinity tyrosine kinase receptors, the flt-1 and the KDR (the human homologue of the murine flk-1 receptor), which are preferentially distributed on vascular endothelial cells but are also known to be present on cardiomyocytes. VEGF also exerts a diverse variety of pleiotropic effects which include an acute cardioprotective effect, and this will be the main focus of this section.

Cardiomyocytes have been demonstrated to produce VEGF mRNA in response to hypoxia or the inhibition of the electron transport chain, an effect which in the human heart is mediated by the stabilization of hypoxia-inducible factor. Luo et al. were the first to demonstrate an acute cardioprotective effect with VEGF pre-treatment. Perfusion of isolated rat hearts with VEGF resulted in improved functional recovery and less cardiac enzyme release following a sustained period of ischaemia–reperfusion injury. However, in this study, the cardioprotecive effect was attributed to a vascular effect, although a direct myocardial effect may have been possible. Both flt-1 and KDR/flk-1 VEGF receptors have been demonstrated to be present in cardiomyocytes, suggesting that VEGF may exert a direct effect on cardiomyocytes. The activation of the VEGF receptor is known to activate signal transduction pathways of the RISK pathway such as MEK1/2-Erk1/2-p90rsk within the cardiomyocyte and PI3K-Akt-eNOS within endothelial cells, which are known to mediate cytoprotection. Therefore, one would surmise that through these signalling cascades, VEGF would have the potential to protect cardiomyocytes from ischaemia–reperfusion injury by acting directly on the cardiomyocyte, although this remains to be demonstrated (Figure 1).

Interestingly, the contribution of VEGF to the endogenous cardioprotective strategy of IPC has been investigated. It has been demonstrated that IPC augments levels of VEGF mRNA in cardiomyocytes in a PKC-ε-dependent manner and that heterozygous mice lacking the VEGF receptors flt-1 and flt-4 sustain greater myocardial ischaemia–reperfusion injury and are resistant to the infarct-limiting effects of IPC, a finding which may be attributed to the downregulation of cardioprotective mediators such as Akt, iNOS, and eNOS. Therefore, further pre-clinical work is required to determine whether VEGF is capable of mediating a direct cardioprotective effect at the level of the cardiomyocyte and to demonstrate whether similar pro-survival signal transduction pathways are involved in conveying the protective signal.

In common with FGF, the clinical application of VEGF has until now been restricted to therapeutic angiogenesis. In this respect, phase 1 clinical studies of patients with refractory ischaemic heart disease examining angiogenic gene therapy using AdVEGF165 or AdVEGF121 have been completed with no safety issues. With regard to the exogenous recombinant VEGF therapy, an intracoronary high-dose of recombinant VEGF (50 ng/kg/min) at day 0 and three subsequent intravenous infusions at days 3, 6, and 9 resulted in significant improvement in anginal symptoms at 120 days in patients with stable coronary artery disease unamanable to revascularization. Whether, a single intracoronary dose of recombinant VEGF as adjuvant reperfusion therapy would be safe and efficacious in patients presenting with a myocardial infarction is unknown.

3.3 Insulin
Insulin is intricately involved with growth and metabolism of the heart and it is this influence on cardiac metabolism during myocardial ischaemia which first attracted its use as part of the glucose–insulin–potassium (GIK) therapy in ischaemic heart disease. There is extensive literature examining the cardioprotective potential of metabolic modulation using GIK therapy in patients presenting with an acute myocardial infarction, and in this respect, the reader is directed to the following reviews. In this section, the primary focus will be on those studies investigating the acute cardioprotective effects of insulin when administered alone (Table 2).

Insulin binds to its specific protein tyrosine kinase receptor (IR), resulting in the autophosphorylation of intracellular
tyrosine residues, leading to the recruitment to the membrane and the phosphorylation of the insulin receptor substrate (IRS), which in turn generates Src homology 2 (SH2)-domain-binding sites for numerous effectors including PI3K and the subsequent activation of Akt.62 In addition, the SH2 domain of the growth factor receptor-bound protein-2 can activate the pre-associated GTP exchange factor Sos, resulting in the activation of the small GTPase, Ras, which then activates the Raf-MEK1/2-Erk1/2 signalling cascade.62

One of the first experimental studies to demonstrate a direct cardioprotective effect with insulin when administered alone was in 1999 by Baines et al.63 In that study, the authors demonstrated that the pre-treatment of rabbit hearts reduced myocardial infarct size in a manner which was dependent on the activation of PI3K and tyrosine kinase.63 In addition, in this study, insulin was demonstrated to limit infarct size even when administered at the onset of myocardial reperfusion, a clinically important time point. A subsequent study by our laboratory demonstrated that insulin was capable of protecting isolated neonatal cardiomyocytes through a potential anti-apoptotic mechanistic pathway.64 Further experimental work has gone on to further delineate the underlying cardioprotective signalling cascades and has identified PI3K-Akt-p70S6K-BAD,65 PI3K-Akt-eNOS,66 and most recently the JAK-STAT pathway upstream of Akt (Table 2 and Figure 1). Interestingly, it was observed that delayed perfusion of the isolated rat heart with insulin after the first 15 min of reperfusion had elapsed did not limit infarct size, suggesting that the cardioprotective end-effector mechanism was mediated in the first few minutes of myocardial reperfusion, the time-window for preventing the opening of the mPTP, a critical mediator of lethal myocardial reperfusion injury.14 Subsequent studies have identified the mPTP as a downstream target of the PI3K-Akt pathway in insulin-induced cardioprotection.12,13

The mechanism through which the JAK-STAT pathway exerts acute cardioprotection at the time of myocardial reperfusion, given its role in gene transcription, is unclear, although it has been speculated that STAT may act directly at the level of the mitochondria to mediate acute cardioprotection (reviewed in reference 68).

Clearly, in the clinical setting, the use of insulin therapy alone as a potential cardioprotectant is not feasible given the risks of hypoglycaemia, although other anti-diabetic agents such as glucagon-like peptide-1, which are capable of stimulating insulin release without causing hypoglycaemia, may be used as potential cardioprotective agents in the clinical setting.69

### 3.4 Insulin-like growth factors

IGF-I and IGF-II were originally identified as somatomedin C in 1956 and multiplication stimulatory action in 1972,70 respectively. They preferentially bind to their respective tyrosine kinase receptors IGF-IR and IGF-IIIR, which are present throughout the body and have been implicated in the regulation of cell growth, differentiation, and survival (reviewed in reference 71). IGF-I is a 72 kDa polypeptide (comprising 70 amino acids), which is produced in the liver in response to growth hormone, and circulates predominantly bound to IGF-binding factors.71

In 1995, Buerke et al.72 were the first group to implicate IGF-I as a cardioprotective agent, demonstrating that pretreatment with this growth factor reduced myocardial necrosis, apoptosis, and neutrophil accumulation. Since then, a variety of experimental studies have confirmed the cardioprotective benefits of IGF-I and IGF-II treatment and have explored the underlying mechanistic pathways (Table 3). Importantly, mice overexpressing IGF-IB (heterozygous) sustained smaller myocardial infarcts, less

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### Table 2: Acute cardioprotection with insulin

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<th>Model</th>
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<td>Baines et al. (1999)</td>
<td>Isolated rabbit heart</td>
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<td>Jonassen et al. (2001)</td>
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<td>Gao et al. (2002)</td>
<td>In vivo perfused rat heart</td>
<td>Treatment at reperfusion with insulin</td>
<td>Reduced MI size</td>
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<td>Gao et al. (2004)</td>
<td>In vivo perfused rat heart</td>
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<td>Juhaszova et al. (2004)</td>
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<td>Fugelstag et al. (2008)</td>
<td>Isolated rat heart and isolated cardiomyocytes</td>
<td>Pre-treatment with insulin</td>
<td>Smaller MI size; improved cell survival</td>
<td>Protection dependent on JAK-STAT pathway upstream of Akt</td>
</tr>
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MI, myocardial infarct.
apoptosis, and less cardiac remodelling at 7 days post-myocardial infarction, confirming the long-term cardioprotective benefits of IGF-I.73

3.4.1 Signal transduction pathways underlying insulin-like growth factor-I cardioprotection
IGF-I exerts its cellular effects by binding to IGF-IR and activating the tyrosine kinase receptor which leads to the autophosphorylation of tyrosine and serine residues and the subsequent phosphorylation of IRS-1 and IRS-2 and downstream signalling pathways such as the PI3K-Akt pathway and MEK1/2-Erk1/2.74 The IRS-1 interacts with and activates PI3K and the guanine-nucleotide exchange factor Grb2/SOS, the latter of which is responsible for recruiting the Ras/Raf/Mek1/2/Erk1/2 kinase cascade. Many of the anti-apoptotic pathways recruited downstream of the PI3K-Akt74–76 and MEK1/2-Erk1/275 pro-survival pathways have been implicated in IGF-I-mediated cardioprotection (Table 3) Crucially, the importance of Akt phosphorylation specifically at the onset of myocardial reperfusion as a mediator of cardioprotection elicited by transgenic overexpression of IGF-I was first observed by Yamashita et al.77

Many of these signal transduction pathways exert anti-apoptotic effects by modulating the balance of anti-apoptotic and pro-apoptotic proteins such as Bcl2 and Bax.78 In addition, the majority of these signal cascades terminate on the mitochondria, resulting in the inhibition of mPTP opening,78 prevention of mitochondrial cytochrome C release,79 maintenance of mitochondrial respiratory production and mitochondrial membrane potential, and less ROS production.79 However, to be useful as a cardioprotective agent, the systemic metabolic effects of IGF-I have to be overcome—in this respect, the selective adenoviral transfection of IGF-I in the myocardium may be promising in cases where myocardial ischaemia can be anticipated, thereby allowing cardioprotection in the absence of raised systemic levels of IGF-I.80

3.4.2 Clinical application of insulin-like growth factor
Cardiovascular risk is doubled in patients lacking GH/IGF-I, suggesting that this growth factor may be important for endogenous cardioprotection (reviewed in reference 81).
In addition, the plasma levels of IGF-I have been reported to be lower in patients presenting with an acute myocardial infarction\(^{82}\) and higher in patients with cardiac failure.\(^{83}\) Importantly, low plasma levels of IGF-I have been linked to worse cardiovascular outcomes.\(^{84,85}\) The haemodynamic effects of a single subcutaneous injection of exogenous IGF-I (60 \(\mu g/kg\)) has been investigated in human volunteers\(^{86}\) and patients with cardiac failure\(^ {87}\) and results in an increase in stroke volume, cardiac output, ejection fraction and reduces systemic vascular resistance, with minimal effect of blood glucose. The inotropic effect exerted by exogenous IGF-I may limit its clinical applicability to patients presenting with an acute myocardial infarction unless an appropriate cardioprotective non-inotropic dose could be found.

### 4. Cardiotrophin-1: a cytokine receptor ligand

CT-1 is a member of the interleukin-6 (IL-6) family of cytokines, which are capable of inducing cardiac hypertrophy by binding to cytokine receptors containing the transmembrane signalling protein gp130. Mice lacking gp130 exhibit severe ventricular hypoplasia, suggesting that gp130-coupled signalling is important for the development of the heart.\(^ {88}\) CT-1 was originally identified by Pennica et al. in 1995\(^ {89}\) as a 21.5 kDa protein capable of inducing hypertrophy in neonatal ventricular cardiomyocytes. One year later, the same research group demonstrated that CT-1 was required for cardiac myocyte maturation and was capable of promoting cell survival in neonatal rat cardiomyocytes subjected to serum deprivation,\(^ {90}\) through an anti-apoptotic pathway mediated by MAPK, Erk1/2.\(^ {91}\) The first study to demonstrate resistance to ischaemia–reperfusion injury with CT-1 treatment was by Stephanou et al.\(^ {92}\) in 1998. In that particular study, CT-1 treatment was associated with the upregulation of heat shock proteins 70 and 90 in neonatal rat cardiomyocytes and it conferred resistance against both simulated ischaemic injury and thermal stress in terms of improved cell survival and less apoptotic cell death.\(^ {92}\)

Since then a variety of experimental studies have reported treatment with CT-1 either prior to myocardial ischaemia or at the onset of myocardial reperfusion using cultured neonatal rat cardiomyocytes (Table 4),\(^ {93,94}\) isolated perfused animal hearts,\(^ {95}\) the intact animal heart, and human atrial tissue\(^ {96}\) (Table 2). Importantly, it was demonstrated that CT-1 was capable of limiting myocardial injury even when administered at the onset of reperfusion, making the transition to clinical therapy more amenable.\(^ {93–95}\) Furthermore, CT-1 has been reported to promote cardiomyocyte survival and reduce apoptotic cell death following non-ischaemic death stimuli such as angiotensin II and hydrogen peroxide.\(^ {97}\)

In addition, CT-1 mRNA has been identified in both the developing and adult animal hearts, and its production is stimulated by hypoxic stress.\(^ {98}\) Following a myocardial infarction, the production of CT-1 mRNA and protein are both enhanced in rat left and right ventricles.\(^ {99}\) These findings may suggest that CT-1 may play a role in endogenous cardioprotection. However, myocardial infarct size was found to be no different in mice lacking CT-1 compared with the wild-type control.\(^ {100}\)

The expression of CT-1 is also increased in the failing animal heart\(^ {101}\) and human heart.\(^ {102}\) Myocardial stretch from cardiac dilatation is responsible for the overexpression of CT-1 in the failing hearts.\(^ {103}\) Interestingly, CT-1 has been detected in human plasma,\(^ {104}\) and its levels are increased in a variety of cardiac conditions including stable and unstable angina,\(^ {105}\) acute myocardial infarction,\(^ {106}\) aortic stenosis,\(^ {107}\) mitral regurgitation,\(^ {108}\) left ventricular hypertrophy,\(^ {109}\) and left ventricular systolic dysfunction.\(^ {110,111}\) Further work is required to determine the pathophysiological significance of these findings.

### Table 4  Acute cardioprotection with cardiotrophin-1

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Treatment regime</th>
<th>Effect</th>
<th>Mechanistic insight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stephanou et al. (1998)(^ {92})</td>
<td>Neonatal rat cardiomyocytes</td>
<td>Pre-treatment with CT-1</td>
<td>Improved cell survival and less apoptotic cell death</td>
<td>First study to demonstrate acute cardioprotection</td>
</tr>
<tr>
<td>Ghosh et al. (2000)(^ {96})</td>
<td>Human right atrial appendage tissue</td>
<td>Acute or chronic (24 h) pre-treatment with CT-1</td>
<td>Less tissue injury following chronic but not acute treatment; CT-1 Ab did not abrogate PC protection</td>
<td>Chronic but no acute protection with CT-1 in human atrial tissue; PC protection not mediated through CT-1</td>
</tr>
<tr>
<td>Brar et al. (2001)(^ {94})</td>
<td>Neonatal rat cardiomyocytes</td>
<td>Pre-hypoxia or at reoxygenation</td>
<td>Improved cell survival and less apoptotic cell death; protection blocked by Erk1/2 inhibition</td>
<td>First study to show protection at the time of reoxygenation; protection is dependent upon Erk1/2 activation</td>
</tr>
<tr>
<td>Brar et al. (2001)(^ {94})</td>
<td>Neonatal rat cardiomyocytes</td>
<td>Pre-hypoxia or at reoxygenation</td>
<td>Improved cell survival and less apoptotic cell death; protection requires Erk1/2, PI3K, and Akt activation</td>
<td>Protection at the onset of reoxygenation and is dependent upon Erk1/2 activation</td>
</tr>
<tr>
<td>Craig et al. (2001)(^ {116})</td>
<td>Neonatal rat cardiomyocytes</td>
<td>Pre-treatment with CT-1</td>
<td>Improved cell survival; protection requires p38, Erk1/2, PI3K, and NFKB activation</td>
<td>Protection dependent upon NFKB activation</td>
</tr>
<tr>
<td>Liao et al. (2002)(^ {95})</td>
<td>Adult rat cardiomyocytes and perfused rat heart</td>
<td>Pre-hypoxia or at reoxygenation</td>
<td>Improved cell survival and less apoptotic cell death; protection is dependent upon Ct-1</td>
<td>Protection in adult cardiomyocytes and perfused heart; protection dependent upon Erk1/2 activation</td>
</tr>
</tbody>
</table>
4.1 Signal transduction pathways underlying cardiotrophin-1 cardioprotection

CT-1 exerts its intracellular effects by binding to a cytokine receptor which comprises a gp130 subunit and a leukaemia inhibitory factor (LIF) receptor subunit β which heterodimerize upon CT-1 binding.112 The dimerization of the CT-1 receptor results in the recruitment of a number of signal transduction pathways (Figure 1). The binding of CT-1 to its cell surface receptor activates receptor-associated Janus family tyrosine kinases (JAKs). The activation of JAK is required for the phosphorylation of a variety of sites on the receptor cytoplasmic domains, which in turn recruit signal relay molecules containing src homology-2 (SH2) and/or phosphotyrosine-binding domains. Signal relay molecules then recruit downstream signalling pathways, including the SH2 domain-containing transcription factors (STAT), the Ras-Raf-MEK1/2-Erk1/2 and the PI3K-Akt signal transduction cascades.113,114 Experimental studies have suggested that the cardiac hypertrophy induced by CT-1 is mediated through the recruitment of the JAK-STAT pathway.91 In contrast, the acute cardioprotection elicited by CT-1 has been associated with the activation of several different signal transduction pathways including (i) the activation of heat shock proteins 70 and 9092; (ii) the phosphorylation of the anti-apoptotic MAPK, Erk1/291,93,94; (iii) the recruitment of the anti-apoptotic signalling cascade PI3K-Akt-BAD93,115; and (iv) the phosphorylation of p38MAPK.116 The mechanism through which these different signal transduction pathways mediate cardioprotection is unclear, although one study has suggested that with respect to p38, Erk1/2, and Akt, CT-1 cardioprotection was demonstrated to be dependent on the downstream nuclear translocation of NFκB.116 However, the acute cardioprotection elicited at the time of re-oxygenation cannot be dependent on gene transcription and may be attributed to the inhibition of mPTP opening, although this remains to be demonstrated with respect to CT-1 cardioprotection. Many of the signalling cascades activate a variety of anti-apoptotic pathways with the inhibition of various pro-apoptotic proteins such as p53, BAX, FAS, BAD and the upregulation of anti-apoptotic proteins such as Bcl2.117 Interestingly, the contribution of the RISK pathway components Erk1/2 MAPK and the PI3K-Akt pathways to CT-1 protection against serum deprivation was demonstrated to be additive, suggesting that these two anti-apoptotic signalling cascades acted in parallel to mediate cellular survival.115 However, in the context of protection from simulated ischaemia–reperfusion injury, both these pro-survival kinase cascades appear to be required as inhibiting either one abolished completely the cardioprotection elicited by CT-1.93

5. Urocortin: a G-protein-coupled receptor ligand

Urocortin was first identified in the rat mid-brain by Vaughan et al. in 1995,118 as a 40 amino acid peptide member of the corticotrophin-releasing hormone (CRH) family, and was given its name because of its homology with fish urotensin and mammalian CRH. It binds to the G-protein-coupled receptors, CRH-receptor 1 (CRH-R1, found predominantly in the brain) and CRH-receptor 2 (CRH-R2, found in the periphery including vascular endothelial cells and the heart), although it acts preferentially at the latter (for comprehensive reviews, please see references 119 and 120). Urocortin has since been found to be present peripherally in various organs, including the heart. Experimental studies have reported peripheral vasodilation in the rat118 and increased cardiac contractility in sheep121 with the systemic administration of urocortin. These haemodynamic effects are blunted in mice lacking CRH-R2122 and underlie their potential utility in congestive cardiac failure (see what follows).

The first study to demonstrate an acute cardioprotective effect with urocortin was by Osoki et al. in 1998,123 who found that pre-treatment with urocortin was able to reduce cell death and LDH release in neonatal rat cardiomyocytes subjected to 6 h hypoxia. Subsequent studies have demonstrated that mRNA expression of urocortin is increased during simulated ischaemia and that it is secreted by neonatal rat ventricular cardiomyocytes during simulated ischaemia and released by isolated perfused rat hearts during sublethal ischaemia (5–20 min),124 suggesting that urocortin may mediate endogenous cardioprotection.125 Interestingly, the cardioprotective effect of bathing the neonatal ventricular cardiomyocytes in hypoxically pre-conditioned medium was abolished by CRH receptor antagonists, suggesting that CRH peptides such as urocortin may mediate the cardioprotective effect of IPC.125 Since these early publications, studies have gone on to confirm that urocortin is able to reduce myocardial infarct size when administered both prior to and at the onset of myocardial reperfusion126,127 (Table 5).

5.1 Signal transduction pathways underlying urocortin cardioprotection

Urocortin exerts its cardioprotective effects by binding to a G-protein-coupled receptor on the cardiomyocyte called the CRH-R2β (in rat heart)128 and CRF-R2α (in the human heart).129 Both urocortin mRNA and CRF-R2α mRNA and receptors have been found in the human heart.130 Experimental studies have demonstrated that the cardioprotective effect is dependent on the activation of pro-survival kinase pathways of the RISK pathway such as the PI3K-Akt127 and the MEK1/2-Erk1/2 cascades.126 The binding of urocortin to its GPCR results in the recruitment to the membrane of PI3K-γ (class IB), leading to the membrane recruitment and activation of Akt.24 In addition, PI3K-γ has the ability to activate mitogen-active protein kinases (MAPKs) such as the MEK1/2-Erk1/2 cascade.131 Importantly, our laboratory132 demonstrated, using the in vivo rat heart, that urocortin administered at the onset of myocardial reperfusion reduced myocardial infarct size through the specific activation of the MEK1/2-Erk1/2 pro-survival signalling pathway. Indeed, it was in this study that the term 'reperfusion injury salvage pathway' was first described.132 The cardioprotective effector mechanisms downstream of these kinase signalling pathways are unclear and appear to converge on the cardiac mitochondria and include the mitochondrial ATP-dependent potassium channel,133-135 PKC activation134 (specifically the PKC-ε isoform at the level of mitochondria),135 reduced mitochondrial phospholipase A135,136 reduced mitochondrial ROS...
potent cardioprotection in a similar manner to urocortin,\textsuperscript{139,140} potentially making them more useful in the clinical setting.

### 5.2 Clinical application of urocortin

Urocortin and its cell surface (CRH-R2α) have been found in the human heart,\textsuperscript{130} and levels of cardiac urocortin are elevated in the presence of dilated cardiomyopathy\textsuperscript{141} and hypertrophic cardiomyopathy.\textsuperscript{142} In patients with mild LV systolic heart failure, plasma levels of urocortin are elevated,\textsuperscript{143} suggesting a potential compensatory mechanism of a failing heart given the known inotropic actions of urocortin. Despite the animal studies reporting an inotropic effect and systemic vasodilation with urocortin I, when

![Table 5 Acute cardioprotection with urocortin](image-url)
investigated in human volunteers and heart failure patients, no haemodynamic effect was actually observed and only increased levels of ACTH and cortisol were demonstrated. It is interesting to note that urocortin is secreted in the human heart and there are specific cell-surface receptors (CRH-R2a) present on the cardiomyocyte, suggesting that the generation of urocortin during myocardial ischaemia may act as an autacoid to endogenously protect the heart in a similar manner to adenosine and bradykinin. Clearly, the use of exogenously administered urocortin as an acutely cardioprotective agent in patients presenting with an acute myocardial infarction will be hampered by the accompanying haemodynamic effects in the case of urocortin II, which has been demonstrated to increase cardiac output, heart rate, ejection fraction and to induce systemic vasodilation, making it ideal therapy for cardiac failure. Urocortin I, which has been reported to have no haemodynamic effects, may be an alternative agent to use in this setting, although its hormonal effects in terms of ACTH production may be problematic.

6. Transforming growth factor-β

TGF-β refers to a superfamily of cytokines comprising nearly 40 members which have been implicated in a diverse variety of cellular processes including cardiac development, vascular fibrosis, apoptosis, inflammation among others (reviewed in references 148 and 149). The TGF-β1 isoform is the one most widely distributed within the cardiovascular system. TGF-β1 is secreted as an inactive latent form which is then activated within the extracellular space. Active TGF-β1 binds to the type II TGF-β receptor (TβR-II), which then recruits and dimerizes with TGF-β receptor type I (TβR-I), forming a heterotrimeric complex which results in the activation of TβR-II and TβR-I intracellular serine–threonine kinases, leading to the stimulation of and the Smad pathway, and the PI3K-Akt and MAPKs such as MEK1/2-ERK1/2 kinase cascades, p38 and JNK.

Initial experimental studies in the early 1990s by Lefer et al. first identified TGF-β1 as a mediator of acute cardioprotection. In the first study, these authors demonstrated that TGF-β1 pre-treatment of the in vivo or ex vivo rat heart reduced myocardial injury, whereas in a subsequent second study, they found that administering TGF-β1 after the onset of myocardial ischaemia and 30 min before myocardial reperfusion reduced myocardial infarct size in the in vivo feline heart subjected to 1.5 h ischaemia and 4.5 h reperfusion. The mechanism of cardioprotection was attributed in these early studies to attenuating two major proponents of myocardial reperfusion injury, namely endothelial dysfunction and neutrophil activation. An additional study reported protective effects at the level of the coronary endothelium. Later studies demonstrated that TGF-β1 was capable of protecting isolated cardiomyocytes (in the absence of endothelium and neutrophils). Our group was the first to report that TGF-β1 could target lethal myocardial reperfusion injury and limit myocardial infarct size when administered at the time of myocardial reperfusion. However, subsequent investigations have described direct cardioprotective effects on the myocardium through the recruitment of intracellular signal transduction pathways including the MEK1/2-Erk1/2 component of the RISK pathway (Table 6). Somewhat surprisingly, Chen et al. found that the pre-treatment of adult rat cardiomyocytes with TGF-β1 improved cell survival following simulated ischaemia–reperfusion injury and this protective effect was associated with less Akt and iNOS phosphorylation but greater levels of eNOS phosphorylation compared with control. Interestingly, the same group also found that the modulation of cardiomyocyte TGF-β1 mediated the cardioprotection elicited by nitric oxide donors although the mechanism linking the two is unclear.

7. Stem cell therapy and growth factors

Accumulating evidence supports a paracrine mode of action in order to explain the beneficial effects of adult stem cell therapy on post-myocardial infarction improvements in cardiac function, with the secretion by adult stem cells of...
cardioprotective and anti-apoptotic growth factors which include FGF-2, VEGF, TGF-β, and IGF-1, contributing to the beneficial effects of stem cell therapy, rather than the engraftment and transdifferentiation of adult stem cells (reviewed in reference 157). Improving the therapeutic efficacy of stem cell therapy in myocardial regeneration following an acute myocardial infarction is a key objective. 'Priming' stem cells by pre-treatment with growth factors has emerged as an innovative treatment strategy for enhancing the cytoprotective abilities of stem cells for myocardial repair. Several growth factors, including FGF-2, 158 IGF-1, 159 and bone morphogenetic protein-2 (BMP-2), 160,161 have been implicated as promoting cardiomyocyte differentiation and the cytoprotective potential in stem cells. Hahn et al. 162 demonstrated that the pre-treatment of rat mesenchymal stem cells with a 'cocktail' comprising FGF-2, IGF-1, and BMP-2 increased gap-junction formation and improved the cytoprotective abilities of the stem cells. Whether these growth factors can be used in a similar manner to improve the therapeutic efficacy of stem cell therapy in the clinical setting of an acute myocardial infarction remains to be seen.

8. Summary and conclusions

There are a number of growth factors which exert a diverse array of cardiovascular effects including the ability to confer acute cardioprotection. Interestingly, many of these growth factors are released by the cardiomyocyte during myocardial ischaemia, suggesting that they may play a role in endogenous cardioprotection. In this respect, genetic ablation studies suggest that some of these growth factors which include FGF-2, VEGF, and urocorin may actually confer endogenous protection against acute ischaemia–reperfusion injury. These particular growth factors protect the heart by activating different receptors including protein tyrosine kinase receptors, cytokines receptors, G-protein-coupled receptors, and serine–threonine receptors present on the cardiomyocyte plasma membrane, which then recruit a set of different molecular pathways leading to cytoprotective abilities of the stem cells. Whether these growth factors can be used in a similar manner to improve the therapeutic efficacy of stem cell therapy in the clinical setting of an acute myocardial infarction remains to be seen.

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Cardioprotective growth factors


