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Mini Review

Role of STAT-1 and STAT-3 in ischaemia/reperfusion injury

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Abstract

Ischaemia/reperfusion (I/R) injury results in the death of irreplaceable cardiac myocytes by a programme cell death or apoptosis. The signal transducers and activators of transcription (STAT) factors function as modulators of cytokine signaling and sensors responding to cellular stress. Interestingly, many studies have demonstrated that although they have a similar structural organization, STAT-1 and STAT-3 have apposing effects on processes such as differentiation or apoptosis. For example, STAT-1 has been shown to induced apoptosis, whilst STAT3 is able protect cardiac myocytes following ischaemia/reperfusion (I/R) injury. Many of the effects of STAT-1 and STAT-3 involve the direct binding to DNA and transcriptional activation of target genes. However, recent studies have shown that for STAT-1 some of its effects appear not to require DNA binding. For example, induction of apoptosis by STAT-1 can be produced by the C-terminal activation domain in the absence of the DNA binding domain. This therefore, appears to involve a co-activator effect in which STAT-1 is recruited to DNA via a DNA-bound transcription factor. In this regard, it is of interest that STAT-1 but not STAT-3 has been shown to interact with p53 and enhance its growth arrest and apoptosis- inducing properties. Hence, the finding that STAT-1 and STAT-3 can modulate the apoptotic programme both by direct DNA binding or via a co-activator mechanism and despite their very similar structures, suggests that these related factors may be therapeutic targets against the damage myocardium following I/R injury. Recently, we reported that the polyphenolic agent epigallocatechin-3-gallate (EGCG), a major constituent of green tea and a potent inhibitor of STAT-1 activation, protects the myocardium against I/R injury.

Keywords: STAT-1 - STAT-3 - heart - ischaemia/reperfusion - heart failure - apoptosis

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Introduction

Protection of the ischaemic myocardium against tissue injury continues to elude basic investigators and clinicians and is therefore still a major objective for the identification of effective strategies for the treatment of ischaemic heart diseases. The limitations of current therapies largely arise from our limited understanding of the molecular events that modulate the severity of myocardial damage during ischaemia/reperfusion (I/R) injury. However, over the past decade it has become clear that the ischaemic myocardium initiates a number of complex signalling pathways that either mediate an adaptive stress-induced protective response, or, if the insult is more severe, activate the cell death pathway which leads to loss of myocytes and compromised cardiac function. Cell death in cardiac myocytes can occur by necrosis or apoptosis, although the relative contribution of the two mechanisms to total myocyte loss remains controversial. Necrosis is a passive and irreversible event associated with swelling and rupture of the cell membrane, leading to release of intracellular contents and consequent inflammation (for review see [1, 2]).

In contrast, the apoptotic programme is an active process whose outcome is determined by a balance between pro- and anti-apoptotic factors; apoptosis is therefore potentially a regulatable process. Apoptosis is characterized by activation of a cascade of cysteine proteases (caspases) which eventually cleave substrates essential for the maintenance of cellular integrity, and is necessarily associated with the ordered fragmentation of DNA (for review see [3, 4]).

Transcriptional modulation of the apoptotic pathway also plays an important role in the initiation of cell death. The related p53 family member, p73 which also exist in several isoforms α, β, γ and ΔN have all been demonstrated in different cell types to regulate whether a cell survives or dies following various stresses (for review see [5]). Most studies on p53 and p73 have focused on models of DNA damage -induced cell death.

Apoptosis in the heart

Ischaemia/reperfusion (I/R) injury in the heart, results in the death and loss of irreplaceable cardiac myocytes. Apoptosis has become increasingly recognized as one of the mechanisms of cell death during I/R injury in the heart (for review see [6]). In human studies, apoptosis has been observed in the infract area of the heart following acute myocardial infarction [7]. A group of cysteine proteases or caspases play a key role as effectors of the apoptotic cell death pathway initiated either by a death receptor pathway via caspase-8 or by agents leading to mitochondrial damage and leakage of cytochrome c leading to activation of caspase-9.

The Fas death receptor pathway has been reported to play an important role in the induction of apoptosis in cardiac myocytes. For example, hearts of mice, which lack functional Fas, show a considerable reduction in cell death following I/R compared to control hearts [8]. Activated caspases have been detected in cardiac myocytes following simulated I/R injury [9]. Moreover, both a specific and a generalized caspase inhibitor (such as ZVAD) abrogated apoptotic cell death in cultured cardiac myocytes and also in the isolated intact heart exposed to simulated I/R [10, 11]. We have recently reported that distinct upstream initiator caspases play a role in apoptotic cell death following ischaemia alone or I/R in cardiac myocytes [10, 11]. We demonstrated by using specific chemical or gene-based caspase inhibitors that caspase-9 plays a key role in neonatal and adult cardiac myocyte apoptosis during simulated ischaemia, whilst caspase-8 induced death occurs during re-oxygenation. We have also substantiated our in vitro studies to demonstrate that the same caspases are activated and induce apoptosis in the isolated intact heart exposed to either ischaemia alone or I/R [11]. Thus, inhibiting the caspase pathway is a potential target for therapeutic intervention during I/R injury.

STATs

Signal transducers and activators of transcription (STAT) factors are a family of cytoplasmic transcription factors that mediate intracellular signaling initiated at cytokine cell surface receptors and transmitted to the nucleus. STATs are activated by phosphorylation on conserved tyrosine and serine residues by the Janus kinases (JAKs) and MAP kinase families respectively, which allow the STATs

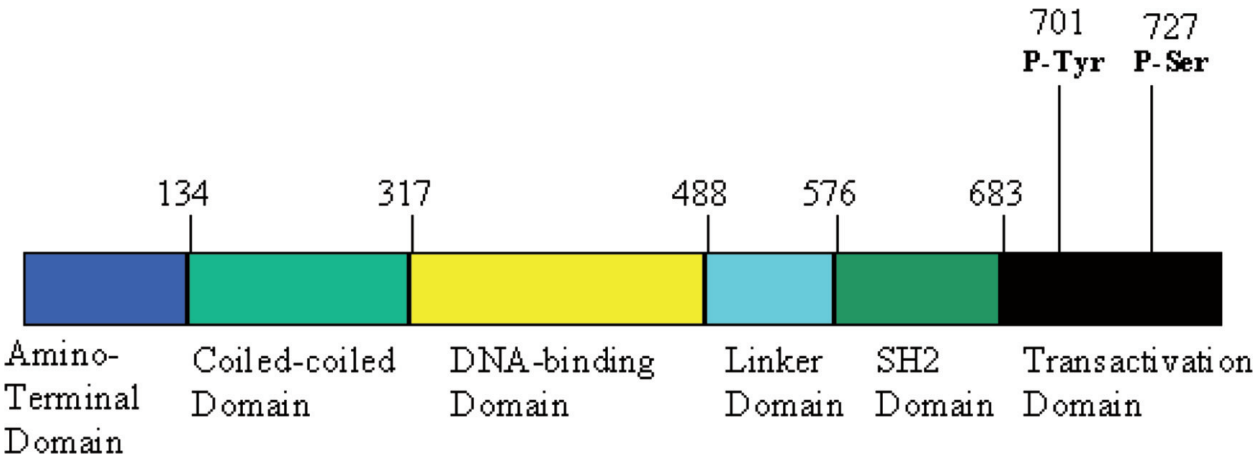

Fig. 1 STAT-1/STAT-3 structure showing the overall domains including the tyrosine (Tyr) and serine (Ser) phosphorylation sites.

to dimerise and translocate to the nucleus and thereby regulate gene expression. The C-terminal domains of STAT proteins all contain a transcriptional transactivation domain (TAD), plus the phosphorylation site for JAKs and MAPK, which are essential for maximal STAT function. At present seven different STAT family members have been characterized and found to be encoded by distinct genes (STAT-1, STAT-2, STAT-3, STAT-4, STAT-5α, STAT-5β and STAT-6). Different STATs are activated by distinct group of cytokines. For example, interferon-γ is a potent activator of STAT-1, whilst the interleukin-6 (IL-6) family members including IL-6, leukemia inhibitory factor (LIF) and cardiotropin-1 (CT-1) primarily activate STAT-3 (for review see [12, 13]).

The overall structure among the STAT proteins (especially STAT-1 and STAT-3) is quite conserved within the coiled-coiled domain (residues114-317), the DNA binding domain (residues 320 and 490), the linker domain (residues 490 and 580) and the SH2 domain (residues 580 and 680). The carboxy-terminal TAD between (residues 680-750 in STAT-1 and STAT-3) is also highly conserved (Fig. 1). In contrast, the amino terminal domain is less conserved among the STATs, suggesting that this part of the protein may be involved in mediating specific responses. Both the coiled-coiled and the SH2 domains are involved in protein-protein interaction.

STAT signalling has also been shown to be negatively regulated by two groups of proteins. One group were identified following the discovery that cytokines which activated STATs were also shown to induce the expression of suppressors of cytokine signalling (SOCs) or STAT-induced STAT inhibitors (SSIs) [14]. These SOCs proteins were shown to bind to the active receptors, which abrogated binding of JAKs and therefore inhibited activation of STAT signalling [15]. Another group of negative STAT activators were identified as nuclear factors that were able to bind to phosphorylated STATs and were named PIAS (protein inhibitors of activated STATs) [16]. PIAS1 was shown to specifically inhibit STAT-1 activation [17], whereas, PIAS3 was a specific inhibitor of STAT-3 [18]. Thus, PIAS1, as well as acting as an inhibitor of STAT-1 activation may also have additional roles in modulating p53 function.

STAT Signaling in the Heart

The STAT activated pathway was first demonstrated to be activated in rat cardiac myocytes following exposure to LIF, resulting in the activation of STAT-3 [19]. Subsequently, it was demonstrated in the intact heart following pressure overload-induced hypertrophy, which leads to the activation of STAT-1 and STAT-3 [20]. Recently it has also been reported that ischaemic preconditioning in the intact heart involves activation of the JAK-STAT pathway. Inhibition of STAT-1 and STAT-3 phosphorylation with the JAK inhibitor AG-490, blocked preconditioning cardio-

protection [21]. Moreover, the classical preconditioning effect is abolished in STAT-3 deficient mice [22]. Ourselves and others have also demonstrated activation of STAT-1 and STAT-3 in the isolated intact heart following I/R injury [23–25]. Pre-treatment with the JAK inhibitor reduced STAT-3 phosphorylation and enhanced apoptosis following I/R. However, cultured cardiac myocytes treated with CT-1, which activates the STAT-3 pathway, enhanced cell survival following exposure to simulated I/R injury and reduced the level of apoptotic cell death [26, 27]. Furthermore, STAT-3 deficient mice are more susceptible to cardiac injury and sensitive to developing heart failure following various stresses to the myocardium [28, 29]. Also, STAT-3 deficient mice have increase apoptotic cardiac myocytes following I/R injury and also much larger infarct sizes compared to wild-type mice [28, 29]. Thus, these studies demonstrate that STAT-3 may be an anti-apoptotic signaling factor in the heart which is able to protect the myocardium following ischaemic injury.

In contrast to STAT-3, STAT-1 plays a role in enhancing apoptotic cell death in cardiac myocytes following simulated I/R injury by inducing the expression of the pro-apoptotic caspase-1, Fas and FasL genes leading to enhanced cardiac cell death [23–25]. Moreover, inhibition of STAT-1 using an anti-sense approach prevented the enhancement of caspase-1, Fas and FasL gene activity in cardiac myocytes exposed to simulated I/R and protected cardiac cells from I/R -induced cell death [23-24]. In addition, we have shown that STAT-1 also inhibits the promoters of genes encoding the anti-apoptotic Bcl-2 and Bcl-x proteins [30]. Hence, STAT-1 activation appears to induce apoptosis in cardiac myocytes by activating pro-apoptotic genes as well as repressing anti-apoptotic genes. We previously examined the mechanism of STAT-1 activity in cardiac myocytes exposed to simulated I/R. We demonstrated that both the tyrosine 701 and serine 727 sites of STAT-1 are phosphorylated in cultured cardiac myocytes and also in the isolated intact heart exposed to I/R [23-24]. However, studies using STAT-1 mutant constructs demonstrated that the induction of Fas and FasL as well as enhanced apoptosis in cardiac myocytes exposed to simulated I/R required the phosphorylation of STAT-1 on serine 727 but not on tyrosine 701 [24]. The phosphorylation of serine 727 of STAT-1 appears to be accomplished by p38 MAPK activated during I/R, since it can be blocked both by the chemical inhibitor SB203580 and by a dominant negative form of MKK6, the upstream activator of p38 MAPK [24].

Although phosphorylation of tyrosine 701 was originally thought to be essential for STAT-1 function, recent studies have shown that some genes can be induced by STAT-1 in a tyrosine 701 independent manner. In addition, serine 727 phosphorylation has also been reported to be critical for the activity of the C-terminal STAT-1 transactivation domain (TAD) to bind to other co-activator molecules such as MCM5 and BRCA1 [31, 32].

Recently we identified a novel protein-protein interaction between STAT-1 and tumour suppressor p53 transcription factor [33]. This association enhances the activity of pro-apoptotic genes in a manner which is dependent on the p53 binding site in their promoters [33]. Similarly, STAT-1 together with p53 was able to enhance the level of apoptosis to a greater extent than either p53 or STAT-1 alone [33]. Interestingly, the STAT-1/p53 association can occur with the C-terminal region of STAT-1 lacking a DNA binding domain paralleling the ability of this domain to enhance apoptosis in cardiac myocytes [33]. In contrast, it has been reported that p53 is able to inhibit the activation of STAT-3 [34] Thus, these studies indicate that STAT-1 but not STAT-3 is able to mediate its effects on gene expression, at least in part, by acting as a coactivator and modulating the functional activity of p53 .

Thus, our studies demonstrate I/R-induced apoptosis requires serine 727 of STAT-1 but not tyrosine 701 in cardiac myocytes. Therefore the activity of the C-terminal TAD of STAT-1 via phosphorylation on serine 727 mediates the effects of cell death in cardiac myocytes exposed to I/R. Support for this comes from our finding that enhanced cell death is observed using a STAT-1 construct encoding only the C-terminal TAD and lacking the DNA binding domain in cardiac myocytes exposed to I/R [35]. Moreover, exchanging serine 727 to a non-phosphorylatable alanine reduced the ability of the isolated C-terminal STAT-1 construct in promoting cell death in cardiac myocytes exposed to simulated I/R. Likewise, cardiac myocytes isolated from mice lacking the N-terminal domain of STAT-1 (amino acids 1-134) but expressing the C-terminal domain were more sensitive to I/R-induced cell death [35]. The isolated

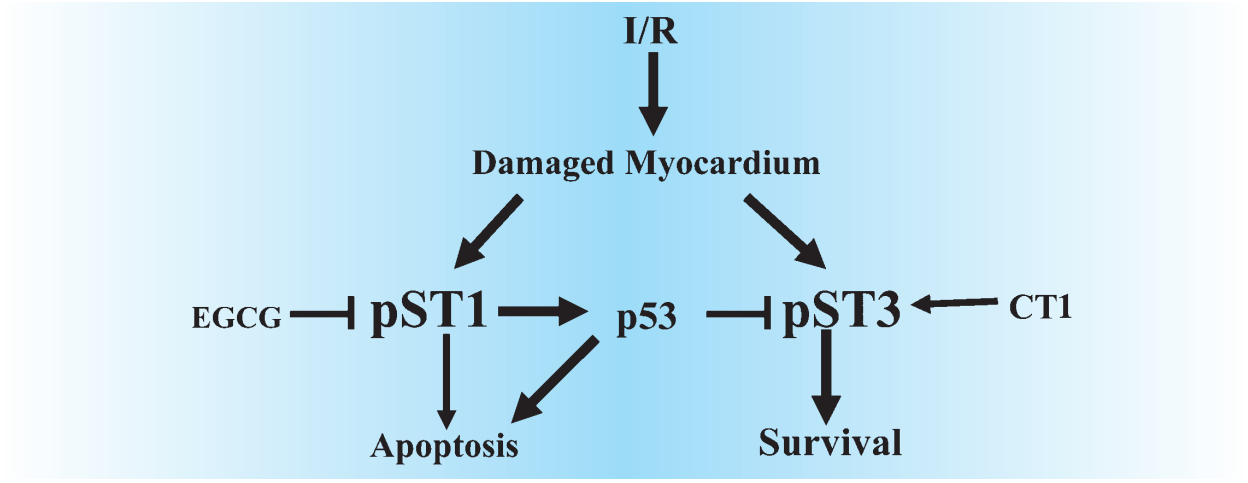

Fig. 2 Hypothetical Pathway demonstrating activation of STAT-1 or STAT-3 in the damage myocardium following exposure of the heart to ischaemia/reperfusion (I/R) injury. This leads to phosphorylation of STAT-1 (pST1) or STAT-3 (pST3) and activation of pro-apoptotic or anti-apoptotic genes. The relative levels of STAT-1 which promotes apoptosis or STAT-3, which promotes survival will determine the fate of cardiac myocytes in the damaged heart. STAT-1 is also able to enhance the apoptotic function of p53 and also p53 is known to inhibit STAT-3 activation, therefore shifting the balance towards apoptotic death. EGCG which is known to inhibit STAT1 activation or CT1 which is able to enhance STAT-3 activation are feasible agents that may reduce injury and promote cell survival in the myocardium following I/R.

intact hearts from these mice exposed to I/R injury had larger infarct size and greater number of TUNEL positive myocytes than control hearts (35).

Interestingly, it has been shown that STAT-1 can be cleaved by caspases such as caspase-3 at position 694 [36]. As mention earlier, we and others have demonstrated that caspases play an active role in apoptotic cell death in cardiac myocytes exposed to I/R [17, 18]. Cleavage of STAT-1 by caspase-3 at position 694 will ultimately release the C-terminal STAT-1 TAD. The N-terminal fragment containing the DNA-binding domain may function as a dominant negative against intact STAT-1 protein, whilst the caspase-mediated generation of the pro-apoptotic C-terminal TAD fragment may be involved in amplifying and perpetuating the apoptotic loop in hearts exposed to ischaemia/reperfusion injury.

STAT-1 and STAT-3: a therapeutic target against the damaged myocardium following I/R injury

The studies mentioned above suggest that modulating STAT-signalling may be an attractive therapy against the damaged myocardium. For example, identification of pharmacological and specific inhibitors of STAT-1 activation may be a feasible way to reduce the apoptotic effects of STAT-1. Recently, it has been reported that the polyphenolic agent epigallocatechin-3-gallate (EGCG), a major constituent of green tea, is a potent inhibitor of STAT-1 phosphorylation and activation [37].

Recently, we have assessed the protective effects of EGCG and green tea extract (GTE) infusion on both cultures of cardiac myocytes and the isolated rat heart. EGCG reduced STAT-1 phosphorylation and protected cardiac myocytes against I/R-induced apoptotic cell death. EGCG also reduced the expression of a known STAT-1 pro-apoptotic target gene, Fas receptor [38]. More interestingly, oral administration of GTE as well as EGCG infusion limited the extent of infarct size and attenuated the magnitude of myocyte apoptosis in the isolated rat heart exposed to I/R injury. The reduction of cell death was associated with improved hemodynamic recovery and ventricular function in the ischemic/reperfused rat heart [38]. This is the first report to show that consumption of green tea is able to mediate cardioprotection and enhance cardiac function during I/R injury. Since GTE-mediated cardioprotection is achieved, at

least in part, through inhibition of STAT-1 activity, we may postulate that a similar action can be implemented in the clinical setting, to minimize STAT-1 activation levels in patients with acute coronary artery disease (CAD).

In contrast to STAT-1, STAT-3 activation would need to be enhanced in order to have any beneficial effects in protecting the damaged myocardium following an ischaemic insult. One feasible way to enhance STAT-3 activation is via a cytokine that is known to primarily induce STAT-3 signaling in the heart such as cardiotropin-1 [26, 27]. CT-1 has previoulsy been shown to protect both neonatal and adult cardiac myocytes against I/R-induced apoptosis [26, 27]. Another way that may be feasible but require testing is a gene therapy approach in which the STAT-3 viral vector expresses a constitutively active form of STAT-3 that is only expressed in the heart and also induced when required.

Conclusion

Myocardial ischaemia/reperfusion injury plays a major role in cell death and the loss of irreplaceable cardiac myocyte, which has been suggested to lead to heart failure. It is clear that STAT-1 plays a critical role in inducing pro-apoptotic genes and apoptosis in cardiac myocytes exposed to I/R, whilst STAT-3 is able to protect against apoptosis in the heart. Therefore the relative levels of activated STAT-1 or STAT-3 may determine the balance between death and survival of cardiac myocytes following I/R induced damage of the myocardium (Fig. 2). Moreover, STAT-1 is known to enhance the functional activity of the p53 pro-apoptotic transcription factor. p53 is also known to inhibit the activation of STAT-3 and so the level of p53 will also shift the relative balance towards cell death rather than survival. Thus, the STAT-1 or the STAT-3 activated pathway are potential therapeutic targets in the prevention of ischaemic heart disease. Agents that are likely to inhibit STAT-1 and not STAT-3 activity and vice versa may lead the ways for developing strategies for therapies that may ultimately lead the way for the prevention of heart failure.

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