



Editorial

Emerging Role of the JAK-STAT Pathway as a Mechanism of Protection Against Ischemia/Reperfusion Injury

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The discovery of ischemic preconditioning (PC) by Murry, Reimer and Jennings¹ in 1986 was a watershed event in the field of ischemic biology. The reasons are obvious. Ischemic PC was the first cardioprotective intervention that was found to be reproducible, having been demonstrated in practically all experimental models tested thus far as well as in humans.^{2–4} Furthermore, ischemic PC proved to be remarkably powerful, such that infarct size could be reduced by as much as 80–90%.²

One of the major developments in the history of PC was the recognition in 1993 that the ischemic stimulus elicits two temporarily distinct (and pathophysiologically different) phases of protection, an early phase that starts almost immediately after the stimulus but is short-lived (1–2 h) and a late phase that requires 12–24 h to appear but is much more sustained (72–96 h).^{2,5–7} Unlike the early phase of PC, which protects only against infarction,⁸ the late phase protects both against stunning and infarction.⁸ Because of this, and because of the vastly (at least 30-fold) longer duration of protection, the late phase of PC may have greater clinical relevance and applicability than the early phase. While the early phase results from rapid post-translational modulation of pre-existing proteins, the late phase

is caused by transcriptional upregulation of cardioprotective genes leading to *de novo* synthesis of proteins that are responsible for the preconditioned phenotype.^{2,7} Hence, despite the similarity of the stimuli that evoke these two responses of the heart, the mechanisms that account for early and late protection are fundamentally different. The last 15 years have witnessed a phenomenal explosion of research into the mechanism of early and late PC, which has resulted in numerous important advances. Nevertheless, the molecular basis of PC, including the signaling pathways responsible for this phenomenon, remains incompletely understood.

The Janus kinases (JAKs) are a family of cytosolic tyrosine kinases that are associated with membrane receptors (e.g., cytokine receptors) and play a critical role in the rapid transduction of signals from the cell surface to the nucleus.⁹ These kinases are named after Janus, the Roman god of gates that was represented as a two-faced divinity. The rationale for this curious nomenclature is that JAKs have “two faces”, consisting of the JH1 domain (the kinase domain) and the JH2 domain (the pseudokinase domain). While the JH1 domain is responsible for the catalytic activity of JAKs, the function of the

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JH2 domain remains unclear, although it has been postulated to be a potential docking site for signal transducers and activators of transcription (STATs).⁹ The first JAK (JAK1) was described in 1989;¹⁰ the JAK-STAT pathway was recognized in 1992 as a functional axis for signal transduction in the context of interferon (IFN) signaling.¹¹ Thus far, four JAKs (JAK1, JAK2, JAK3 and Tyk2) have been identified in mammals. The binding of a ligand (such as a cytokine) to its receptor causes dimerization of the receptor to which JAKs are associated. This, in turn, enables transphosphorylation and activation of two adjacent JAKs. The activated JAKs then phosphorylate the tyrosine motifs in the cytoplasmic tail of the receptor, which creates docking sites for the Src homology 2 domains of the STAT proteins.⁹ STATs are a unique class of transcription factors with seven known members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6), which play a critical role in regulating the expression of multiple genes.^{12,13} Upon recruitment to the receptor signaling complex, STATs are tyrosine phosphorylated by JAKs; this enables them to form homodimers or heterodimers that translocate to the nucleus and bind to γ -IFN activation site (GAS) motifs, resulting in gene transcription.^{12,13}

An extensive body of work has demonstrated that the JAK-STAT pathway plays a crucial role in the expression of stress-responsive genes in a variety of cells and organs.^{9,13} Although this pathway was initially implicated in immune cell function, it is now well established that it operates in many other cell types. In the heart, experimental evidence suggests a role of the JAK-STAT pathway in cardiac hypertrophy^{14,15} and apoptosis.¹⁶ Activation of JAK1 and STAT3 has been reported during permanent coronary occlusion in rats,^{17,18} and a possible antiapoptotic role of these molecules has been conjectured.¹⁸ A recent report, however, suggests that angiotensin type 1 receptors induce activation of STAT5A and STAT6 in the setting of a 30-min coronary occlusion followed by reperfusion, resulting in increased apoptosis.¹⁹ Proapoptotic and antiapoptotic effects of STAT1 and STAT3, respectively, have been demonstrated in cultured neonatal cardiac myocytes subjected to anoxia, metabolic inhibition, and acidosis.¹⁶ However, until recently very little was known about the function of JAKs and STATs in the setting of myocardial ischemia-reperfusion, and virtually nothing was known regarding their involvement in PC.

Xuan *et al.*²⁰ have recently identified an important role of the JAK-STAT pathway in the development of cardioprotection associated with the late phase

of ischemic PC. Using a mouse model of late PC against infarction, these investigators demonstrated that exposure of the heart to brief episodes of ischemia/reperfusion induces rapid tyrosine phosphorylation of JAK1 and JAK2 (but not of JAK3 or Tyk2). Western immunoblotting and electrophoretic mobility shift assays further demonstrated a rapid translocation of STAT1 and STAT3 from the cytosol to the nucleus concomitant with increased tyrosine phosphorylation and DNA-binding activity of these transcription factors. The activation of STAT1 and STAT3 was highly selective, as none of the other five members of the STAT family (STAT2, STAT4, STAT5A, STAT5B, and STAT6) were affected by ischemic PC. Administration of the JAK inhibitor AG-490 prior to the PC ischemia abrogated both the activation of JAK1/JAK2 and that of STAT1/STAT3.

Pre-treatment with AG-490 also abolished the upregulation of inducible nitric oxide synthase (iNOS) protein and activity and the delayed protection against infarction induced by late PC. This study²⁰ established an essential role of JAK-STAT signaling in the adaptation of the heart to stress and identified JAK1, JAK2, STAT1, and STAT3 as the specific elements involved in the recruitment of iNOS and in the attending acquisition of ischemic tolerance. However, since the molecular mechanisms underlying the early and late phases of ischemic PC differ,^{7,21} the role of the JAK-STAT pathway in the early phase of ischemic PC remains unknown.

In this issue of the *Journal*, Hattori *et al.*²² make an important contribution by demonstrating that JAK2 and STAT3 are causally involved in the early phase of ischemic PC. Using isolated working rat hearts subjected to global ischemia, the authors found increased tyrosine phosphorylation of JAK2 and STAT3 immediately after the ischemic PC stimuli as well as 2 hours after reperfusion following 30 minutes of global ischemia in the PC group. Protection against ischemia/reperfusion injury by early PC was documented by an improved functional recovery, reduced myocardial infarct size, and decreased number of apoptotic cardiomyocytes. Interestingly, ischemic PC induced upregulation of the antiapoptotic gene bcl-2 and downregulation of the proapoptotic gene bax, a finding that has significant implications for the protective effects of PC. The JAK inhibitor AG-490 abolished JAK2 and STAT3 phosphorylation, ablated the protection against infarction and apoptosis, and blocked the upregulation of bcl-2 and the downregulation of bax. Taken together, the data by Hattori *et al.* point to JAK2 and

STAT3 as important signaling elements responsible for the antiapoptotic and cardioprotective effects associated with early PC.

This study expands our understanding of JAK-STAT signaling in myocardial ischemia. The finding that JAK2 and STAT3 are rapidly activated by ischemic PC confirms the results of Xuan *et al.*²⁰ and extends them to the setting of global ischemia. The effects of AG-490 support a cause-and-effect relationship between these molecular events and the cardioprotection afforded by early PC. This is a novel finding, as the involvement of JAKs and STATs in the early phase of protection afforded by ischemic PC had not heretofore been reported. Thus, the work by Hattori *et al.* complements that of Xuan *et al.*,²⁰ in that the latter reveals an obligatory role of JAK-STAT signaling in the late phase of PC whereas the former indicates an essential role in the early phase. Taken together, these studies^{20,22} identify a heretofore unrecognized function of the JAK-STAT axis as an innate mechanism of protection against myocardial ischemia/reperfusion injury, thereby revealing a novel function of this pathway.

Activation of JAK1/2 and STAT1/3 leads to delayed protection, at least in part, via upregulation of iNOS.²⁰ How does mobilization of the JAK-STAT pathway mitigate ischemia/reperfusion injury during the early phase of PC? This is perhaps the most intriguing question raised by the work of Hattori *et al.*²² JAK-STAT signaling has thus far been implicated in relatively slow responses that involve transduction of signals from the extracellular milieu to the nucleus, activation of gene transcription, and synthesis of new proteins.^{9,13} This paradigm is hardly applicable to early PC—a response that develops almost immediately (in a matter of minutes) after the stimulus.² It therefore seems unlikely that the increased synthesis of iNOS protein following activation of JAK-STAT signaling demonstrated by Xuan *et al.*²⁰ would contribute to the early phase of PC. Similarly, the finding of Hattori *et al.* that JAK-STAT activation potentiates a pro-survival pathway (i.e., upregulation of bcl-2 and downregulation of bax) is likely to have implications for the mechanism of delayed, rather than early, cardioprotection. Further work will obviously be needed to elucidate the mechanism whereby JAK-STAT signaling contributes to early PC.

Besides the general concept that JAK2 and STAT3 participate in the cardioprotective effects of early PC, another interesting aspect of the article by Hattori *et al.* is the analysis of early PC-induced signaling events in relation to protection

against cardiomyocyte apoptosis. Although the modulation of bcl-2 and bax by myocardial ischemia has been well documented (e.g.²³), the exact signaling events governing the altered expression of these genes during myocardial ischemia/reperfusion remain largely enigmatic. Using a semiquantitative approach to measure RNA expression, these authors demonstrated a decrease in bax and an increase in bcl-2 expression in the ischemic PC group. These changes were reversed, although not completely in the case of bax, by administration of AG-490, which also blocked the tyrosine phosphorylation of JAK2 and STAT3. Although definitive proof of the involvement of STAT3 in the transcriptional modulation of these genes will require documentation of increased STAT3 DNA-binding activity to the binding sites in the bcl-2 and bax promoters, these authors do suggest an involvement of STAT3 in transmitting antiapoptotic signals in cardiomyocytes in the setting of ischemia/reperfusion.

Despite rapid progress, much remains to be learned regarding the signaling pathways that underlie the early and late phases of ischemic PC. The complexity of this phenomenon seems to grow in direct proportion to the amount of work aimed at elucidating it. Nevertheless, given the fact that ischemic or pharmacologic PC remains the most reproducible and effective cardioprotective mechanism identified to date, and considering the potentially widespread applications of exploiting this innate cardioprotective mechanism for the mitigation of myocardial ischemia/reperfusion injury in patients with coronary artery disease, it is essential that the vigorous effort mounted in the last few years to unravel the molecular basis of preconditioning be carried into the future with the goal of fully deciphering the cascade of events responsible for acquisition of ischemic tolerance. Only then will it be possible to design with confidence PC-mimetic pharmacologic or genetic strategies that can faithfully recapitulate the preconditioned phenotype in an effective, controlled, and non-hazardous manner.

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