Role of Mitogen-Activated Protein Kinases in Ischemia and Reperfusion Injury

The Good and the Bad

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There is growing evidence that multiple mitogen-activated protein (MAP) kinases are activated during ischemia and/or reperfusion and may contribute to the structural and functional changes after myocardial ischemia. A new study by Yue et al1 in this issue of Circulation Research attempts to address the role of MAP kinases in ischemic myocardium and suggests that ERK1/ERK2 is part of a “survival” pathway whereas p38 and JNK mediate a “death” pathway in the ischemic myocardium.

Myocyte loss during the acute stage of myocardial infarction involves both apoptotic and necrotic cell death.2–4 Therefore, it is reasonable to think that the balance of cell survival and death is critical during the pathological evolution of postischemic cardiac dysfunction. Recently, many scientists seeking to elucidate the intricate relationship between signal transduction and this balance of survival and death in ischemic myocardium have focused on MAP kinases.

MAP Kinases

MAP kinases are highly conserved serine/threonine kinases that are activated in response to a wide variety of stimuli including growth factors, G protein–coupled receptors, and environmental stresses.5 Consequently, they play a role in numerous cell functions including growth and proliferation. The MAP kinases themselves require dual phosphorylation on a Thr-X-Tyr motif to become active. Three major MAP kinase cascades have been extensively studied in the heart: extracellular signal–regulated kinases (ERK1 and ERK2), c-Jun N-terminal kinases (JNK1 and JNK2), and p38 kinases, of which the p38\(\alpha\) and p38\(\beta\) isoforms are found in the heart.5 Recently, a fourth MAP kinase member, big MAPK-1 (BMK1, also known as ERK5), has been identified in cardiac tissue (Figure).6

JNK and p38: Proapoptotic Bad Guys?
The pathways regulated by p38 and JNK contribute importantly to apoptosis. The mechanisms by which p38 and JNK induce apoptosis are largely cell and stimulus specific. For example, apoptosis in response to deprivation of growth factors principally involves the activation of p38 rather than JNK.7 In some cells,8 JNK-mediated apoptosis has been shown to involve ubiquitination and degradation of p53, which may be regulated by JNK-mediated phosphorylation of p53.9 However, p53 does not appear to be important for hypoxia-induced apoptosis in cardiac myocytes. Webster et al9 reported that cell death mediated by hypoxia combined with acidosis was independent of p53 because there was no significant difference between apoptosis occurrence in hearts from wild-type mice and p53 knockout mice. Thus, the mechanism for JNK-induced apoptosis in the heart remains unexplained.

The role of p38 in myocyte apoptosis is puzzling because p38 can also mediate cardiac hypertrophy. One of the ways that p38 can induce apoptosis is through its effect on cyclin D1 expression during cell cycle. It has been demonstrated that the coexpression of MKK3 along with p38 inhibits mitogen-induced cyclin D1 expression.10 However, evidence implies a protective role for p38 during ischemia: p38 phosphorylates MAPKAPK2, which in turn phosphorylates HSP27.11 Activation of this pathway is cytoprotective, and overexpression of HSP27 confers protection against ischemia in myocytes.12,13 Protection by ischemic preconditioning also appears to be dependent on p38 activation,14 and direct activation of p38 and JNK by anisomycin is cardioprotective.14 Activation of different p38 isoforms may explain these findings; p38\(\alpha\) may be proapoptotic, whereas p38\(\beta\) may be antiapoptotic in rat neonatal ventricular myocytes.15 In summary, the current data for p38 and JNK suggest multiple roles in ischemia, reperfusion, preconditioning, and hypertrophy. Defining their precise roles and interaction will require cardiac-specific p38 and JNK knockout mice.

ERK1 and BMK1: Antiapoptotic Good Guys?
Although ERK1/ERK2 was the first MAP kinase described, a closely related member BMK1 (ERK5) has recently been identified.16,17 Both ERK1/ERK2 and BMK1 activation are protective against apoptotic cell death. Among the substrates of ERK, special attention should be given to p90 ribosomal S6 kinase (p90RSK), which is a ubiquitous and versatile mediator of ERK signal transduction.18 Essential functions of p90RSK include (1) regulation of gene expression via phosphorylation of transcription factors including c-Fos and cAMP-response element-binding (CREB) protein; (2) regulation of protein synthesis by phosphorylation of polyribosomal proteins and glycogen synthase kinase-3; and (3) stimulation of Na+-H+ exchanger by phosphorylating serine 703 of...
NHE-1. Recently Bonni et al. and Tan et al. reported that p90RSK phosphorylated the proapoptotic protein BAD at serine 112, which specifically suppressed BAD-mediated apoptosis. These findings suggest that p90RSK and ERK promote cell survival by both inhibiting components of the cell death machinery (eg, BAD) and increasing transcription of prosurvival genes (eg, CREB) (Figure).

In the study by Yue et al., the authors concluded that ERK1/ERK2 is important for myocyte survival in ischemic myocardium based on the effect of the MEK-1 inhibitor PD98059. However, the use of PD98059 as a selective MEK1/MEK2 inhibitor is not as safe as previously thought. Recently, this compound was found to inhibit the activation of BMK1 as well as ERK. MEK5 and BMK1 are highly expressed in cardiac myocytes. Importantly, BMK1 has also been proposed to be ant apoptotic based on results of MEK5 inhibition. MEK5-dependent BMK1 activation results in the phosphorylation of MEF2A and MEF2C, transcription factors that belong to the myocyte enhancer factor-2 (MEF2) family, which are important regulators of cardiac gene expression. Because BMK1 can also be activated in the ischemic myocardium, it will be necessary to evaluate the role of this MAP kinase in PD98059-induced survival.

**Apoptosis, Necrosis, and Cardiac Function**

Yue et al. chose apoptosis as their endpoint of cell death as opposed to necrosis. Until recently, it was accepted that irreversible cell death in the ischemic myocardium was due to necrosis. However, evidence has accumulated over the last decade indicating that cardiac myocytes are capable of undergoing apoptosis and that this process may play a role in ischemic injury of the heart. Interestingly, it appears that both ischemia and reperfusion are required to induce apoptosis; ischemia alone is insufficient as shown in both the present and previous studies. However, the degree to which apoptosis contributes to myocardial damage is a hotly debated issue that may be due in part to the techniques used for detecting apoptosis. Studies have shown that assays thought to be apoptosis specific can also detect necrotic cells, thus potentially overestimating the number of apoptotic myocytes. The fact that apoptosis occurs over several hours to days (as opposed to necrosis, which can occur in minutes) casts doubts as to whether this form of death contributes to the acute functional change associated with infarction.

The present study challenges this concept because Yue et al. showed significant improvement of cardiac function after global ischemia within only 1 hour of reperfusion using SB203580, a p38 inhibitor. In a similar study the authors also found that apoptosis could be detected after only 1 hour of reperfusion, and this was reversed by SB203580. These data would indicate that the improvement of cardiac function is directly correlated with the prevention of apoptosis. However, SB203580 also reduced infarction so that p38 may have other effects on cardiac function unrelated to apoptosis. We propose that the posttranslational effects of MAP kinases, such as phosphorylation of NHE-1 and BAD, contribute to the protective effects in early cardiac function recovery, perhaps to a greater extent than alterations in gene expression and apoptosis (Figure).


**Key Words:** ischemia/reperfusion ■ mitogen-activated protein kinase ■ apoptosis ■ cardiomyocyte
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doi: 10.1161/01.RES.86.6.607

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/86/6/607

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