FR167653 diminishes infarct size in a murine model of myocardial ischemia-reperfusion injury

Masaki Yada, MD^a
Akira Shimamoto, MD, PhD^{a,b}
Craig R. Hampton, MD^b
Albert J. Chong, MD^b
Hiroo Takayama, MD^b
Christine L. Rothnie, BS^b
Denise J. Spring, PhD^b
Hideto Shimpō, MD, PhD^a
Isao Yada, MD, PhD^a
Timothy H. Pohlman, MD^b
Edward D. Verrier, MD^b

Objective: During myocardial ischemia-reperfusion injury, p38 mitogen-activated protein kinase is activated. We examined the effect of a highly specific inhibitor of p38 mitogen-activated protein kinase, FR167653, in an experimental model of regional myocardial ischemia-reperfusion.

Methods: CD-1 mice received FR167653 intraperitoneally 24 hours before 30 minutes of transient occlusion of the left anterior descending artery, followed by 120 minutes of reperfusion. The p38 mitogen-activated protein kinase activation and kinase activity were determined by Western blotting with monoclonal antibodies for the phosphorylated form of p38 mitogen-activated protein kinase or its substrate, activating transcription factor 2. Nuclear factor κ B activity was measured by detecting translocation of nuclear factor κ B to the nucleus. The expression of inflammatory cytokines was measured by ribonuclease protection assay.

Results: Pretreatment of mice with FR167653 before myocardial ischemia-reperfusion resulted in a reduction in p38 mitogen-activated protein kinase phosphorylation ($P = .018$), an inhibition of p38 mitogen-activated protein kinase activity ($P = .047$), a smaller amount of nuclear factor κ B ($P = .001$), and a decrease in the expression of inflammatory cytokines (tumor necrosis factor α : $P = .023$, interleukin 1β : $P = .038$, monocyte chemoattractant protein 1: $P = .0001$) in the heart and the development of a significantly smaller infarct ($P = .0069$) relative to hearts from mice treated with vehicle alone. Activation of c-Jun N-terminal kinase and extracellular signal-regulated kinase were observed after myocardial ischemia-reperfusion without inhibition by FR167653.

Conclusion: We conclude that FR167653 selectively inhibits p38 mitogen-activated protein kinase activation and activity during regional myocardial ischemia-reperfusion injury and efficaciously reduces infarct size (by 73.6%). Thus p38 mitogen-activated protein kinase inhibition may have a role in the treatment of myocardial ischemia-reperfusion.

From the Department of Thoracic & Cardiovascular Surgery,^a Mie University School of Medicine, Tsu, Japan, and the Division of Cardiothoracic Surgery,^b Department of Surgery, University of Washington School of Medicine, Seattle, Wash.

Supported in part by the National Institutes of Health grant 5R01HL061762 and a Grant-in-Aid for Scientific Research (No. 14370408) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Received for publication Oct 21, 2003; revisions requested Jan 16, 2004; accepted for publication Feb 2, 2004.

Address for reprints: Akira Shimamoto, MD, PhD, Division of Cardiothoracic Surgery, Department of Surgery, University of Washington School of Medicine, 1959 NE Pacific St, Box 356310, Seattle, WA 98195-6310 (E-mail: jj6jdv@u.washington.edu).

J Thorac Cardiovasc Surg 2004;128:588-94
0022-5223/\$30.00

Copyright © 2003 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2004.02.007

Myocardial ischemia-reperfusion injury is a major risk factor for patients undergoing treatment for cardiac disease, including percutaneous coronary angioplasty, coronary artery bypass grafting, heart and lung transplantation, and other cardiac procedures that require cardiopulmonary bypass.¹⁻⁴ Although the pathophysiology of myocardial ischemia-reperfusion injury remains largely unknown, activation of mitogen-activated protein kinases (MAPKs), including p38 MAPK, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), are early events. Both p38 MAPK and JNK are associated with cellular responses to stress, whereas ERK activity is associated with growth and development processes in the cell. Once activated, the MAPKs phosphorylate and activate other intracellular mediators, resulting in the transcription of genes that encode such proteins as adhesion molecules, proinflammatory mediators, procoagulant molecules, and vasoactive substances.⁵⁻¹⁰ This sequence of events may progress to irreversible tissue injury caused by an acute inflammatory response.¹¹⁻¹⁴

Recently, FR167653* has been shown to be a specific p38 MAPK inhibitor without affecting cyclooxygenase (COX) or other MAPKs.¹⁵ It competes with adenosine triphosphate (ATP) at the ATP-binding site of only p38 MAPK.¹⁵

We hypothesize that myocardial ischemia-reperfusion stress activates MAPKs, with subsequent activation of nuclear factor (NF) κ B, resulting in enhanced cytokine expression associated with myocardial ischemia-reperfusion injury. Further, we postulate that specific inhibition of p38 MAPK with FR167653 will attenuate myocardial ischemia-reperfusion injury through inhibition of NF- κ B. In this study, we evaluated the effect of FR167653 on myocardial ischemia-reperfusion injury in mice.

Materials and Methods

Animals and Experimental Design

Male CD-1 mice weighing 20 to 25 g (Charles River Laboratory, Inc, Wilmington, Mass) were divided into three groups: (1) the FR167653 group in which 200 μ L of FR167653 (2 mg/kg dissolved in saline solution; Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan) was administered intraperitoneally 24 hours before 30 minutes of ischemia followed by 120 minutes of reperfusion, (2) a vehicle group in which 200 μ L of saline solution was administered intraperitoneally 24 hours before myocardial ischemia-reperfusion, and (3) a control group in which 200 μ L of saline solution was administered intraperitoneally 24 hours before sham operation.

A mouse model of in situ regional myocardial ischemia-reperfusion was used as previously described elsewhere.¹⁶ In brief, the mice were intubated and placed under mechanical ventilation after

undergoing general anesthesia with pentobarbital sodium (100 mg/kg, intramuscularly). A lateral thoracotomy was performed to expose hearts, and myocardial ischemia was produced by ligating the left anterior descending coronary artery (LAD) with a 7-0 silk suture. After 30 minutes of ischemia, the occlusive snare was released to initiate reperfusion for 120 minutes. Sham-operated control mice underwent the same surgical procedures except that the suture that was passed under the LAD was not tied. Molecular analysis end points were as follows: 5 minutes of reperfusion for MAPK activation, p38 MAPK activity and I κ B α phosphorylation; 60 minutes of reperfusion for NF- κ B activity; and at the completion of reperfusion for messenger RNA (mRNA) to assess cytokine expression. Hearts were rapidly explanted and the left ventricle was dissected free, rinsed in 0.9% saline solution, snap-frozen in liquid nitrogen, and then stored at -80°C until subsequent analysis, whereas area at risk and infarct size were determined at the completion of reperfusion as previously described elsewhere.¹⁶

All animals were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals" (<http://www.nap.edu/catalog/5140.html>) and also with the Guideline of the Animal Care and Use Committee of the University of Washington.

Western Blotting Assay for Phosphorylation of MAPKs and I κ B α

Whole-cell protein, extracted from frozen tissue samples with ice-cold lysis buffer (Cell Signaling Technology, Inc, Beverly, Mass), were stored at -80°C until the time of assay. A 20- μ g portion of whole-cell protein was loaded onto 15% dodecylsulfate-polyacrylamide electrophoretic gels and transferred to polyvinylidene difluoride membranes. The membranes were immunoblotted with primary antibodies in PhosphoPlus p38 MAPK (Thr180/Tyr182), JNK (Thr180/Tyr182), ERK (Thr180/Tyr182), and I κ B α (Ser32) Antibody Kits (1:1000; Cell Signaling Technology). Immunoreactivity was quantitated with enhanced chemiluminescence and determined with densitometry (NIH Image 1.62; National Institutes of Health, Bethesda, Md). The ratio of phospho-MAPK to total MAPK, or phospho-I κ B α , immunoreactivity was determined for each sample, and the results are expressed as fold-increase with respect to control.

p38 MAPK Activity Assay

The p38 MAPK activity was measured with the p38 MAPK Assay Kit (Cell Signaling Technology) combining immunoprecipitation, kinase assay, and immunoblotting. The results are shown as the immunoreactivity of phosphorylated activating transcription factor (ATF) 2 (Thr71), detected with enhanced chemiluminescence and determined with densitometry (NIH Image 1.62). Results are expressed as multiples of increase in activation with respect to control.

Electrophoretic Mobility Shift Assays for NF- κ B Activity

Myocardial nuclear proteins from frozen tissue samples were isolated as previously described.¹⁶ A 10- μ g portion of nuclear protein was incubated in a binding reaction with a ^{32}P -end-labeled, double-stranded oligonucleotide containing the human and rodent consensus NF- κ B binding sequence, 5'-AGTTGAGGGGACTT-TCCCAGGC-3' (Promega Co, Madison, Wis) and subjected to

*1-[7-(4-fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo (5-1-c)(1,2,4) triazin-2-yl]-2-phenylethanedione sulfate monohydrate.

Figure 1. FR167653 blocks myocardial ischemia-reperfusion. CD-1 mice were subjected to myocardial ischemia-reperfusion injury, and area at risk (AAR) as a percentage of left ventricle (LV) and infarct size as a percentage of area at risk were determined as described in Materials and Methods section for control mice that received vehicle alone (cross-hatched bars) or vehicle with FR167653 (FR, 2 mg/kg, black bars). Values represent mean $\pm$ SD of 5 animals; asterisk indicates $P < .01$ versus vehicle.

electrophoresis in native 6% nondenaturing polyacrylamide gels. Densitometry was performed with NIH Image 1.62. Results are expressed as fold-increase in activation with respect to control.

Ribonuclease Protection Assay

Total RNA was isolated from frozen tissue samples by guanidium thiocyanate-phenol-chloroform method. RNA from each tissue was evaluated with RPA III Ribonuclease Protection Assay Kit (Ambion, Inc, Austin, Tex) and customized mouse cytokines template (Riboquant Multi-Probe Template Set; BD Biosciences Pharmingen, San Diego, Calif) according to the manufacturer's protocol. Densitometric analysis was done with NIH Image 1.62. Results are expressed as multiples of increase with respect to control after the amount of mRNA for each cytokine was normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA.

Statistical Analysis

All data are expressed as mean $\pm$ SD. The significance of the difference between group means was analyzed by analysis of variance with post hoc comparisons by Scheffé protected least-significant difference test. All statistical analyses were done with StatView 6.0 software (SAS Institute, Inc, Cary, NC).

Results

Effect of p38 MAPK Inhibition on Myocardial Infarct Size After Ischemia-Reperfusion

The ratio of area at risk to left ventricle did not differ between vehicle-treated and FR167653-treated groups. Pretreatment with FR167653 significantly reduced infarct size within the area at risk by 73.6% relative to vehicle pretreat-

Figure 2. Effects of FR167653 (FR) on myocardial MAPK activation after ischemia-reperfusion. Western blotting analyses of phospho-JNK and total JNK (A), ERK (B), and p38 MAPK (C) expressions. D, Phosphorylation of MAPKs. Values represent mean $\pm$ SD of 5 animals; asterisk indicates $P < .001$ versus control; dagger indicates $P < .05$ versus vehicle.

ment (FR167653 vs vehicle: $11.4\% \pm 6.8\%$ vs $43.2\% \pm 3.3\%$, $P = .0069$; Figure 1).

Myocardial MAPK Activation After Ischemia-Reperfusion

Phosphorylation of JNK was significantly increased in both groups after myocardial ischemia-reperfusion compared with the control group (FR167653: 6.97 ± 1.38 -fold increase, $P < .001$, vehicle: 6.39 ± 1.08 -fold increase, $P < .001$); there was no significant difference between FR167653 and vehicle groups. Phosphorylation of ERK was also significantly increased in both groups after myocardial ischemia-reperfusion relative to the control group (FR167653: 2.48 ± 1.28 -fold increase, $P < .001$, vehicle: 2.50 ± 1.08 -fold increase, $P < .001$), and there was no difference between those that received FR167653 pretreatment versus pretreatment with vehicle. Myocardial ischemia-reperfusion significantly increased phospho-p38

Figure 3. Effects of FR167653 (FR) on myocardial p38 MAPK activity after ischemia-reperfusion. **A**, Western blotting analysis of phospho-ATF-2 expression after immunoprecipitation and kinase assay for p38 MAPK activity. **B**, p38 MAPK activities. Values represent mean $\pm$ SD of 5 animals; asterisk indicates $P < .001$ versus control; dagger indicates $P < .05$ versus vehicle.

MAPK relative to control (FR167653: 5.68 ± 1.46 -fold increase, $P < .001$, vehicle: 10.17 ± 2.77 -fold increase, $P < .001$). In contrast to JNK and ERK activation, FR167653 pretreatment significantly reduced the activation of p38 MAPK ($P = .018$ vs vehicle; Figure 2). Myocardial ischemia-reperfusion also significantly increased p38 MAPK activity, which was measured directly by detecting phosphorylation of a p38 MAPK substrate, ATF-2, in both groups relative to the control group (FR167653: 6.51 ± 3.36 -fold increase, $P < .001$, vehicle: 9.74 ± 3.56 -fold increase, $P < .001$). Pretreatment of mice with FR167653 before myocardial ischemia-reperfusion, however, significantly attenuated p38 MAPK activity relative to vehicle-treated animals ($P = .047$, Figure 3).

Effect of p38 MAPK Inhibition on Myocardial NF- κ B Activation After Ischemia-Reperfusion

Relative to the control group, in vehicle-pretreated animals, there was a significant rise in NF- κ B activation, as determined by assay of NF- κ B translocation to the nuclear fraction (2.00 ± 0.20 -fold increase, $P < .001$). Pretreatment with FR167653, however, abrogated this activation (1.18 ± 0.12 -fold increase, $P = .001$ vs vehicle). Phosphorylation of I κ B α , which is required for NF- κ B activation, was significantly suppressed by FR167653, consistent with inhibition by FR167653 of p38 MAPK-mediated NF- κ B activation (FR167653: 0.98 ± 0.23 -fold increase, $P = .028$ vs vehicle, vehicle: 3.99 ± 2.01 -fold increase, $P < .05$ vs control; Figure 4).

Figure 4. Effects of p38 MAPK inhibition by FR167653 (FR) on myocardial NF- κ B activation after ischemia-reperfusion. **A**, Electrophoretic mobility shift assay of NF- κ B activation. **B**, Western blotting analysis of phospho-I κ B α expression as indirect indicator of NF- κ B activation. **C**, NF- κ B activities and phosphorylation of I κ B α . Values represent mean $\pm$ SD of 5 animals; asterisk indicates $P < .001$ versus control; dagger indicates $P < .05$ versus vehicle.

Effect of p38 MAPK Inhibition on Myocardial Inflammatory Cytokine Gene Expression After Ischemia-Reperfusion

Myocardial ischemia-reperfusion stress significantly increased mRNA levels of tumor necrosis factor (TNF) α , interleukin (IL) 1β , and monocyte chemotactic protein (MCP) 1 in vehicle-pretreated mice (TNF- α : 1.56 ± 0.27 -fold increase, $P < .05$ vs control, IL- 1β : 3.46 ± 1.14 -fold increase, $P < .01$ vs control, MCP-1: 7.46 ± 0.48 -fold increase, $P < .001$ vs control). Pretreatment with FR167653 significantly attenuated this inflammatory cytokine response (TNF- α : 0.65 ± 0.18 -fold increase, $P = .023$ vs vehicle, IL- 1β : 1.57 ± 0.14 -fold increase, $P = .038$ vs vehicle, MCP-1: 2.84 ± 0.77 -fold increase, $P = .00015$ vs vehicle; Figure 5). FR167653 had no effect on expression of lymphotactin; macrophage inflammatory protein 1 α , 1 β , and 2; RANTES (regulated on activation, normal T-cell expressed and secreted), T-cell activation gene 3, or eotaxin (data not shown).

Discussion

This study demonstrates that myocardial ischemia-reperfusion injury in a mouse model causes rapid phosphorylation and activation of p38 MAPK and JNK, subsequent NF- κ B activation, cytokine expression in the ischemia-reperfusion-injured myocardium, and ultimately infarction. A limitation of this study is that we measured only myocardial infarction

Figure 5. Effects of p38 MAPK inhibition by FR167653 (FR) on myocardial inflammatory cytokine gene expression after ischemia-reperfusion. **A**, Ribonuclease protection assay of TNF- α , IL-1 β , MCP-1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, internal control) mRNA expressions. **B**, Vertical axis denotes multiplicity of increase relative to control after amount of mRNA for each cytokine was normalized to that of glyceraldehyde-3-phosphate dehydrogenase mRNA. Values represent mean $\pm$ SD of 5 animals; asterisk indicates $P < .05$ versus control; dagger indicates $P < .05$ versus vehicle.

as an end point of regional ischemia-reperfusion injury. Although possible to a limited degree in mice, acquisition of myocardial functional data is exceedingly difficult and potentially unreliable. We therefore relied on infarct size as our end point measurement of regional myocardial ischemia-reperfusion injury. These cellular events, with the exception of JNK and ERK activation, were suppressed by pretreatment of mice with FR167653, which specifically blocks p38 MAPK activation by competing with ATP at the ATP-binding site of only p38 MAPK.

Previous studies have used SB203580 as a p38 MAPK inhibitor.¹⁷⁻¹⁹ The actions of SB203580, however, are not confined to selective inhibition of p38 MAPK. SB203580 has been reported to inhibit thromboxane synthase, COX-1 and -2,²⁰ and JNK,²¹ all of which may directly affect injury after myocardial ischemia-reperfusion. This nonspecific ac-

tion of SB203580 may be the reason for the conflicting observations that have been reported regarding the role of p38 MAPK in myocardial ischemia-reperfusion injury.

The p38 subfamily of MAPKs consists of four isoforms (α , β , γ , and δ); only p38 α and p38 β have been detected in the cardiac tissue.²² MAPK kinases (MKKs) selectively activate p38 MAPK in different cell types and exhibit isoform specificity: MKK3 activates only α and γ isoforms, whereas MKK6 can activate α , β , and γ isoforms.²² Recent studies revealed a different role for p38 α and β isoforms in apoptotic responses and cell survival: a negative role for p38 α and positive role for p38 β .^{10,23} One possible explanation for these divergent functions of p38 MAPK isoforms is that they mediate phosphorylation of different downstream substrates that have different roles in apoptosis. Ischemia-reperfusion and ischemic preconditioning may differentially activate p38 MAPK isoforms, accounting for the controversies in previous studies. Ischemia-reperfusion has been shown to activate p38 α .^{5,24,25} Myocytes that ectopically expressed p38 α and p38 β showed activation of p38 α during sustained simulated ischemia, whereas the p38 β isoform was deactivated.²⁶ Moreover, in rat heart cells expressing wild-type p38 α , injury was reduced by p38 MAPK inhibition, suggesting that the selective inhibition of the p38 α isoform of the p38 MAPK pathway may underlie the mechanism responsible for anti-ischemic effects observed after p38 MAPK inhibition.¹⁰ The p38 β isoform appears to play a positive role in the protection against apoptotic cell death.²³

FR167653 is a specific inhibitor for p38 MAPK; it inhibits p38 α strongly but not specifically.¹⁵ In our study, however, FR167653 significantly suppressed phosphorylation of both the α and β p38 isoforms. Some studies have reported that p38 α is mainly phosphorylated under prolonged ischemia and reperfusion conditions, such as our model; however p38 β has been phosphorylated in transient ischemia, including ischemic preconditioning.^{23,26-28} This shows that a specific p38 MAPK inhibitor, although not specific for the p38 α isoform, was effective in preventing myocardial ischemia-reperfusion injury.²⁹

Myocardial ischemia-reperfusion injury is thought to result from an intense local inflammatory response involving cellular (neutrophil, endothelial cell, and monocyte) activation and elaboration of inflammatory mediators including cytokines, chemokines, and adhesion molecules. These inflammatory mediators in turn are capable of further activating multiple cell types and thus propagating an inflammatory response. Our results indicate that in murine hearts subjected to ischemia-reperfusion, MAPKs and inflammatory transcription factors become activated, resulting in elaboration of multiple inflammatory mediators and myocardial tissue destruction. NF- κ B promotes transcription of many genes that encode proteins involved in inflammation.

NF- κ B activation is involved in myocardial ischemia-reperfusion injury and also ischemic preconditioning.³⁰ However, these previous results may not be directly extrapolated to the entire pathway in myocardial ischemia-reperfusion injury, particularly between MAPKs and NF- κ B, and therefore the roles of cardiac MAPKs remain to be elucidated in these clinically relevant pathophysiologic conditions. Proinflammatory mediators correlated with myocardial ischemia-reperfusion, such as inflammatory cytokines, chemokines, and some growth factors, have been shown to activate MAPK pathways.³¹ In addition, TNF- α and IL-1 β can be generated by p38 MAPK-dependent pathways.³² The focus of our study was on evaluation of p38 MAPK as a possible therapeutic target to minimize myocardial ischemia-reperfusion injury.

Ischemic heart disease and acute myocardial infarction represent a major therapeutic challenge. Mammalian cells respond to ischemia with activation of numerous cell-signaling cascades that can eventually lead to irreversible damage of the myocardium and cell death. Our studies have found that ischemia-reperfusion induce distinct regulation of specific MAPK cascades, with noted differences in the intensity and time course of their activation, as well as interspecies differences.³³ Activation of multiple parallel MAPK pathways may be important in the heart's response to cellular stress.⁵ In this study, increased phosphorylations of p38 MAPK, JNK, and ERK were confirmed immediately after reperfusion.

We have previously evaluated the toxicity of long-term treatment in a murine model of FR167653 (2 and 10 mg/[kg $\cdot$ d] intramuscularly) daily for 18 weeks by measurement of serum levels of glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, creatine kinase, alkaline phosphatase, blood urea nitrogen, and creatinine along with histopathologic assessment of brain, heart, lung, liver, and kidney. There were no abnormalities in any of these parameters at the completion of the 18-week study (data not shown). Because of the potential clinical applicability suggested by our results, additional pharmacologic studies will be important.

In conclusion, we have shown in a mouse model that myocardial ischemia-reperfusion causes rapid phosphorylation and activation of MAPKs, with subsequent NF- κ B activation, which results in cytokine expression and myocardial ischemia-reperfusion injury. Pretreatment with FR167653, which specifically and potently inhibits p38 MAPK, reduces NF- κ B activation and inflammatory cytokine expression, decreasing myocardial tissue injury. These data show that p38 MAPK plays a central role in the molecular events that underlie myocardial ischemia-reperfusion injury. Accordingly, inhibition of p38 MAPK activity may be a therapeutic target in the prevention of inflammatory cytokine mediated tissue destruction seen with such

hyperinflammatory disorders as myocardial ischemia-reperfusion.

We thank Fujisawa Pharmaceutical Co, Ltd, for their generous gift of FR167653.

References

1. Verma S, Fedak PW, Weisel RD, Butany J, Rao V, Maitland A, et al. Fundamentals of reperfusion injury for the clinical cardiologist. *Circulation*. 2002;105:2332-6.
2. Weisel RD. Myocardial stunning after coronary bypass surgery. *J Card Surg*. 1993;8:242-4.
3. Meyers BF, Lynch J, Trulock EP, Guthrie TJ, Cooper JD, Patterson GA. Lung transplantation: a decade of experience. *Ann Surg*. 1999;230:362-71.
4. Verrier ED, Morgan EN. Endothelial response to cardiopulmonary bypass surgery. *Ann Thorac Surg*. 1998;66:S17-9.
5. Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, Ben-Levy R, Ashworth A, et al. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. *Circ Res*. 1996;79:162-73.
6. Knight RJ, Buxton DB. Stimulation of c-Jun kinase and mitogen-activated protein kinase by ischemia and reperfusion in the perfused rat heart. *Biochem Biophys Res Commun*. 1996;218:83-8.
7. Ma XL, Kumar S, Gao F, Loudon CS, Lopez BL, Christopher TA, et al. Inhibition of p38 mitogen-activated protein kinase decreases cardiomyocyte apoptosis and improves cardiac function after myocardial ischemia and reperfusion. *Circulation*. 1999;99:1685-91.
8. Mackay K, Mochly-Rosen D. An inhibitor of p38 mitogen-activated protein kinase protects neonatal cardiac myocytes from ischemia. *J Biol Chem*. 1999;274:6272-9.
9. Hreniuk D, Garay M, Gaarde W, Monia BP, McKay RA, Cioffi CL. Inhibition of c-Jun N-terminal kinase 1, but not c-Jun N-terminal kinase 2, suppresses apoptosis induced by ischemia/reoxygenation in rat cardiac myocytes. *Mol Pharmacol*. 2001;59:867-74.
10. Martin JL, Avkiran M, Quinlan RA, Cohen P, Marber MS. Antiischemic effects of SB203580 are mediated through the inhibition of p38 α mitogen-activated protein kinase: evidence from ectopic expression of an inhibition-resistant kinase. *Circ Res*. 2001;89:750-2.
11. Fan H, Sun B, Gu Q, Lafond-Walker A, Cao S, Becker LC. Oxygen radicals trigger activation of NF- κ B and AP-1 and upregulation of ICAM-1 in reperfused canine heart. *Am J Physiol Heart Circ Physiol*. 2002;282:H1778-86.
12. Lakshminarayanan V, Lewallen M, Frangogiannis NG, Evans AJ, Wedin KE, Michael LH, et al. Reactive oxygen intermediates induce monocyte chemotactic protein-1 in vascular endothelium after brief ischemia. *Am J Pathol*. 2001;159:1301-11.
13. Nossuli TO, Frangogiannis NG, Knuefermann P, Lakshminarayanan V, Dewald O, Evans AJ, et al. Brief murine myocardial ischemia-reperfusion induces chemokines in a TNF- α -independent manner: role of oxygen radicals. *Am J Physiol Heart Circ Physiol*. 2001;281:H2549-58.
14. Kacimi R, Karlner JS, Koudssi F, Long CS. Expression and regulation of adhesion molecules in cardiac cells by cytokines: response to acute hypoxia. *Circ Res*. 1998;82:576-86.
15. Takahashi S, Keto Y, Fujita T, Uchiyama T, Yamamoto A. FR167653, a p38 mitogen-activated protein kinase inhibitor, prevents *Helicobacter pylori*-induced gastritis in Mongolian gerbils. *J Pharmacol Exp Ther*. 2001;296:48-56.
16. Hampton CR, Shimamoto A, Rothnie CL, Griscavage-Ennis J, Chong A, Dix DJ, et al. HSP70.1 and -70.3 are required for late-phase protection induced by ischemic preconditioning of mouse hearts. *Am J Physiol Heart Circ Physiol*. 2003;285:H866-74.
17. Nakano A, Cohen MV, Critz S, Downey JM. SB 203580, an inhibitor of p38 MAPK, abolishes infarct-limiting effect of ischemic preconditioning in isolated rabbit hearts. *Basic Res Cardiol*. 2000;95:466-71.
18. Mocanu MM, Baxter GF, Yue Y, Critz SD, Yellon DM. The p38 MAPK inhibitor, SB203580, abrogates ischaemic preconditioning in

- rat heart but timing of administration is critical. *Basic Res Cardiol*. 2000;95:472-8.
19. Maulik N, Yoshida T, Zu YL, Sato M, Banerjee A, Das DK. Ischemic preconditioning triggers tyrosine kinase signaling: a potential role for MAPKAP kinase 2. *Am J Physiol*. 1998;275(5 Pt 2):H1857-64.
 20. Borsch-Haubold AG, Pasquet S, Watson SP. Direct inhibition of cyclooxygenase-1 and -2 by the kinase inhibitors SB 203580 and PD 98059. SB 203580 also inhibits thromboxane synthase. *J Biol Chem*. 1998;273:28766-72.
 21. Clerk A, Sugden PH. The p38-MAPK inhibitor, SB203580, inhibits cardiac stress-activated protein kinases/c-Jun N-terminal kinases (SAPKs/JNKs). *FEBS Lett*. 1998;426:93-6.
 22. Sugden PH, Clerk A. "Stress-responsive" mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. *Circ Res*. 1998;83:345-52.
 23. Wang Y, Huang S, Sah VP, Ross J Jr, Brown JH, Han J, et al. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem*. 1998;273:2161-8.
 24. Marais E, Genade S, Huisamen B, Strijdom JG, Moolman JA, Lochner A. Activation of p38 MAPK induced by a multi-cycle ischaemic preconditioning protocol is associated with attenuated p38 MAPK activity during sustained ischaemia and reperfusion. *J Mol Cell Cardiol*. 2001;33:769-78.
 25. Barancik M, Htun P, Strohm C, Kilian S, Schaper W. Inhibition of the cardiac p38-MAPK pathway by SB203580 delays ischemic cell death. *J Cardiovasc Pharmacol*. 2000;35:474-83.
 26. Saurin AT, Martin JL, Heads RJ, Foley C, Mockridge JW, Wright MJ, et al. The role of differential activation of p38-mitogen-activated protein kinase in preconditioned ventricular myocytes. *FASEB J*. 2000;14:2237-46.
 27. Tamura K, Sudo T, Senfleben U, Dadak AM, Johnson R, Karin M. Requirement for p38 α in erythropoietin expression: a role for stress kinases in erythropoiesis. *Cell*. 2000;102:221-31.
 28. Otsu K, Yamashita N, Nishida K, Hirotsu S, Yamaguchi O, Watanabe T, et al. Disruption of a single copy of the p38 α MAP kinase gene leads to cardioprotection against ischemia-reperfusion. *Biochem Biophys Res Commun*. 2003;302:56-60.
 29. Gao F, Yue TL, Shi DW, Christopher TA, Lopez BL, Ohlstein EH, et al. p38 MAPK inhibition reduces myocardial reperfusion injury via inhibition of endothelial adhesion molecule expression and blockade of PMN accumulation. *Cardiovasc Res*. 2002;53:414-22.
 30. Morgan EN, Boyle EM Jr, Yun W, Griscavage-Ennis JM, Farr AL, Canty TG Jr, et al. An essential role for NF- κ B in the cardioadaptive response to ischemia. *Ann Thorac Surg*. 1999;68:377-82.
 31. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature*. 1994;372:739-46.
 32. Lee JC, Kumar S, Griswold DE, Underwood DC, Votta BJ, Adams JL. Inhibition of p38 MAP kinase as a therapeutic strategy. *Immunopharmacology*. 2000;47:185-201.
 33. Ravingerova T, Barancik M, Strniskova M. Mitogen-activated protein kinases: a new therapeutic target in cardiac pathology. *Mol Cell Biochem*. 2003;247:127-38.

Bound volumes available to subscribers

Bound volumes of ***The Journal of Thoracic and Cardiovascular Surgery*** are available to subscribers (only) for the 2004 issues from the Publisher, at a cost of \$134.00 for domestic, \$165.85 for Canadian, and \$155.00 for international subscribers for Vol 127 (January-June) and Vol 128 (July-December). Shipping charges are included. Each bound volume contains a subject and author index.

The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. Payment must accompany all orders. Contact Elsevier Inc., Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887, USA; phone 800-654-2452 or 407-345-4000.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.