Parecoxib Inhibits Apoptosis in Acute Myocardial Infarction Due to Permanent Coronary Ligation But Not Due to Ischemia–Reperfusion

Fadi N. Salloum, PhD, Nicholas N. Hoke, BS, Ignacio M. Seropian, MD, Amit Varma, MD, Evan D. Ownby, MD, Jon-Erik Houser, Benjamin W. Van Tassell, PharmD, and Antonio Abbate, MD

Purpose: Myocardial ischemia induces cyclooxygenase 2 (COX-2) expression. We evaluated the effects of parecoxib, a COX-2 inhibitor, in 2 different mouse models of myocardial ischemia: permanent left coronary artery ligation (PI) and transient ligation (30 minutes ischemia) followed by reperfusion (I/R).

Methods: Forty adult male Institute of Cancer Research mice underwent PI (n = 24) or I/R (n = 16), followed by randomization to parecoxib (0.75 mg/kg intraperitoneal daily) or normal saline for 7 days.

Results: Parecoxib significantly reduced apoptosis [0.8% vs. 3.4% (saline), $P < 0.001$] and 7-day mortality [0% vs. 57% (saline), $P = 0.040$] in the PI group but showed no benefit in the I/R group. Parecoxib-treated mice also exhibited greater fractional shortening in the PI group [22% vs. 14% (saline), $P = 0.045$] but not in the I/R group. Parecoxib did not affect infarct size in either group.

Conclusions: COX-2 may play a pivotal role in mediating apoptosis in the ischemic peri-infarct myocardium that is not reperfused after infarct.

Key Words: apoptosis, cyclooxygenase 2 inhibitor, ischemia–reperfusion, myocardial infarction, parecoxib

(*J Cardiovasc Pharmacol*™ 2009;53:495–498)

INTRODUCTION

Cyclooxygenase 2 (COX-2) is an enzyme that catalyzes the conversion of arachidonic acid to prostaglandin H_2. Recent interest has arisen regarding the role of COX-2 in ischemic heart disease. COX-2 is not expressed in the normal myocardium but it is expressed upon ischemia.[1] Myocardial COX-2 may be multifunctional, as various reports describe beneficial and negative effects from COX-2 activity. The reported beneficial effects of COX-2 include late phase preconditioning, protection against myocardial stunning, and myocardial infarction.[2] Conversely, COX-2 inhibitors may reduce apoptosis and improve ventricular function after acute myocardial infarction (AMI) in rats and mice,[3–6] whereas they may negatively affect ischemic preconditioning.[7] These discrepancies may be partially attributed to differences in ischemic models, as most in vivo studies used a model of permanent coronary ligation, whereas most in vitro studies used models of transient ischemia followed by reperfusion.

The aim of this study was to evaluate the effects of parecoxib, the only available injectable selective COX-2 inhibitor (administered at a dose corresponding to that shown to control pain in experimental studies, 0.75 mg/kg), on infarct size, apoptosis, and ventricular remodeling in 2 different models of AMI in the mouse: permanent coronary ligation [permanent ischemia (PI)] and transient coronary ligation (30 minutes) followed by reperfusion [ischemia/reperfusion (I/R)].

METHODS

Surgical Procedures

All animal experiments were conducted under the guidelines on the humane use and care of laboratory animals for biomedical research published by National Institutes of Health (No. 85-23, revised 1996). The Institutional Animal Care and Use Committee of Virginia Commonwealth University approved the study. Adult male outbred Institute of Cancer Research mice (age, 10 weeks and weight, 26–38 g) were purchased from Harlan Laboratories (Boston, MA). Twenty-four mice underwent permanent coronary ligation and 16 underwent transient coronary ligation. For permanent coronary ligation, mice were orotracheally intubated under anesthesia (pentobarbital 50–70 mg/kg) and placed in the right lateral decubitus position. Mice then underwent left thoracotomy, pericardiectomy, and ligation of the proximal left coronary artery. For transient coronary ligation, mice underwent the same surgical procedure, but the coronary artery ligation was released after 30 minutes of ischemia, before closure of the thorax. Sham operations were performed for both models of ischemia wherein animals underwent the same surgical

Received for publication February 14, 2009; accepted March 25, 2009.
From the Division of Cardiology, Pauley Heart Center, Virginia Commonwealth University, Richmond, VA.
Supported in part by funds from the Thomas F. Jeffress and Kate Miller Jeffress Trust award, the Societa' Italiana di Cardiologia training grant, and the American Heart Association Beginning Grant-in-Aid to Dr. A. Abbate.
The drug (parecoxib) used in the experiments was kindly provided by Pfizer Italia (Rome, Italy).
None of the investigators have potential conflict of interests inherent to this study.
Reprints: Dr. Antonio Abbate, MD, Division of Cardiology, Pauley Heart Center, Virginia Commonwealth University, 1200 East Broad Street—West Hospital, 10th Floor, East Wing, Room 1041, PO Box 980281, Richmond, VA 23298-0281 (e-mail: aabbate@mcvh-vcu.edu).

procedure without coronary artery ligation. All surgical procedures were performed by the same operator (F.N.S.).

Echocardiography

All animals underwent transthoracic echocardiography (TTE) under light anesthesia (pentobarbital 30–50 mg/kg) before surgery and again at day 7 before sacrifice. The chest was shaved and the mice were placed supine. Doppler echocardiography was performed with the Vevo770 imaging system (Visual Sonics, Inc, Toronto, Ontario, Canada) and a 30-MHz probe. The transducer was positioned on the left anterior side of the chest. The heart was first imaged in the 2-dimensional mode in the short axis view of the left ventricle. M-mode images were then obtained at the level of the papillary muscles below the mitral valve tip according to the American Society of Echocardiography recommendations.[8] The M-mode cursor was positioned perpendicular to the anterior and posterior wall to measure left ventricular (LV) end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), systolic infarct wall thickness, diastolic infarct wall thickness, and noninfarct systolic and diastolic wall thickness. LV fractional shortening (FS) was calculated as follows: FS = 100*(LVEDD − LVESD)/LVEDD. Ejection fraction was calculated with the Teichholz formula. The investigators performing and reading the echocardiogram were blinded to the treatment.

Treatment

Immediately after surgery, animals were randomly assigned to receive a soluble COX-2 inhibitor, parecoxib (0.75 mg/kg intraperitoneal daily), for 7 days or the same volume of saline (500 μL).

Infarct Size and Apoptosis

On day 7, after echocardiography, the heart was explanted and fixed in a 10% formalin solution for at least 48 hours. Transverse sections of the median third of the left ventricle were taken and processed. Infarct size was measured as scar area in sections stained with Masson trichrome (Sigma-Aldrich, St. Louis, MO) by computer morphometry using a BIOQUANT imaging software and was expressed as a percentage of the left ventricle. Apoptosis was defined by costaining for TUNEL (DNA fragmentation; Oncor, Gaithersburg, MD) and activated caspase-3 [cleaved caspase-3 (Asp 175) antibody from Cell Signaling Technology, Beverly, MA; dilution 1:50]. The detailed protocol is published elsewhere.[5] The peri-infarct area was defined as the zone bordering the infarct where viable myocardium was prevalent and reparative fibrosis was only marginal.[5] The apoptotic rate (AR) was expressed as the number of apoptotic cardiomyocytes divided by all cardiomyocytes per field. AR in the peri-infarct regions was calculated on 5 random fields, which virtually cover the entire peri-infarct area. The investigators performing the cell count were blinded to the treatment.

Statistical Analysis

Statistical analysis was performed with the SPSS 11.0 package for Windows (SPSS, Inc, Chicago, IL). Continuous variables are expressed as mean and standard error. One-way analysis of variance was used to compare means between multiple (>2) groups with the post hoc 2-sided Dunnett test to specifically compare the between-subject effects with controls in each group. The *t* test for unpaired data was used to compare means between 2 groups only. Random-effects analysis of variance for repeated measures was used to compare preintervention and postintervention echocardiographic parameters between the 4 different groups with the post hoc 2-sided Dunnett test to specifically compare the between-subject effects (parecoxib and saline groups). Kaplan–Meier survival curves were constructed, and the log-rank test was used to evaluate for significant differences between groups. Unadjusted 2-tailed probability values are reported throughout, with statistical significance set at the 0.05 level. All the authors have read and agree to the article as written.

RESULTS

There were no differences in any of the results between both sham-operated groups (PI and I/R protocol), and these groups were pooled together for subsequent analyses.

Survival

None of the sham-operated animals died during the protocol. In the PI group, all animals treated with parecoxib (10 of 10) survived to day 7, whereas only 6 of 14 animals (43%) survived in the PI saline-treated group ($P < 0.05$ vs. parecoxib). In the I/R group, no deaths were observed in either treatment arms (Fig. 1).

Cardiac Remodeling

Figure 2 (panels A–C) shows cardiac remodeling and ventricular function at 7 days in the different surgical groups. In the PI group, parecoxib-treated mice experienced smaller deteriorations in LVFS at 7 days versus saline-treated mice (22.2 ± 3.3% vs. 12.7 ± 2.5%, $P = 0.045$). These mice also experienced a trend toward smaller dilations in LVESD (3.8 ± 0.3 vs. 4.7 ± 0.1 mm, $P = 0.078$) and LVEDD (4.78 ± 0.4 vs. 5.3 ± 0.1 mm, $P = 0.09$). In the I/R group, parecoxib did not produce any significant changes versus saline in LVEDD (4.0 ± 0.1 vs. 4.2 ± 0.2 mm, $P = 0.8$), LVESD (2.6 ± 0.3 vs. 3.1 ± 0.2 mm, $P = 0.2$), or LVFS (33.0 ± 2.6% vs. 28.1 ± 2.6%, $P = 0.2$).

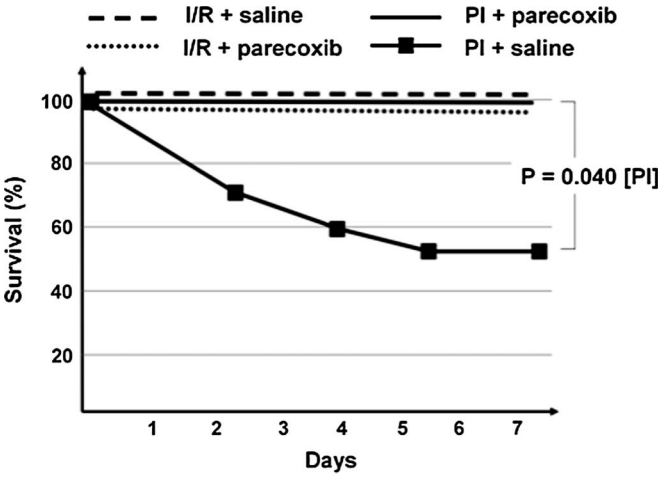

FIGURE 1. Effects of parecoxib on survival in mice after PI or I/R.

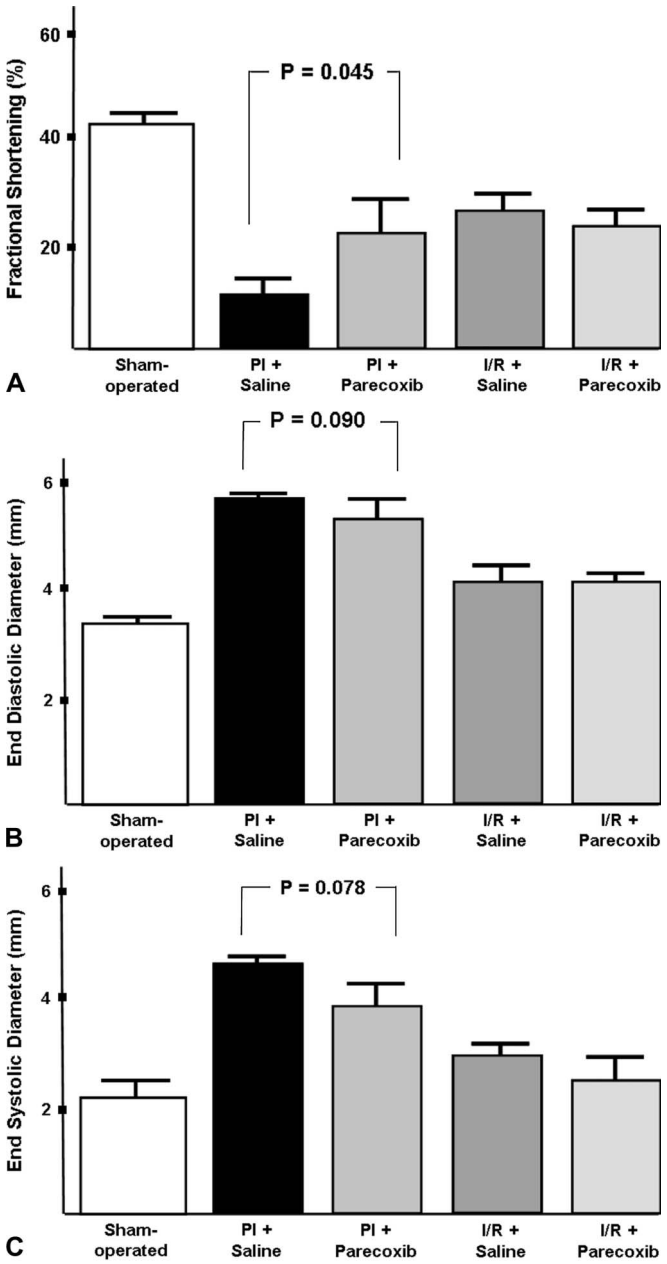

FIGURE 2. Effects of parecoxib on cardiac remodeling and ventricular function in mice after PI or I/R.

Infarct Size and Apoptosis

Figure 3 shows infarct size in all the treatment groups at 7 days. As expected, I/R was associated with a significant reduction in infarct size independent of the treatment arm ($P < 0.001$). Parecoxib did not affect infarct size independently of the model used (PI vs. I/R). Figure 4 shows the AR at 7 days. Apoptosis in the peri-infarct myocardium was significantly reduced by reperfusion (1.1 ± 0.2% vs. 3.4 ± 0.4% for I/R and PI, respectively, $P < 0.001$). Treatment with parecoxib significantly reduced ARs in the PI group but did not affect apoptosis in the I/R group.

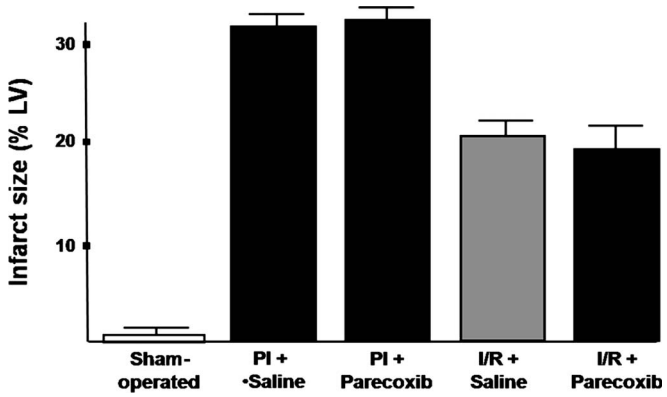

FIGURE 3. Effects of parecoxib on infarct size in mice after PI or I/R.

DISCUSSION

This is the first study to compare the effects of a selective COX-2 inhibitor, parecoxib, in two different mouse models of AMI characterized by different durations of ischemia and occurrence of reperfusion. Although the model of ischemia–reperfusion may resemble the clinical scenario of patients being subjected to percutaneous or surgical reperfusion, reperfusion is often suboptimal and residual or recurrent ischemia often occurs. A combination of ischemia/reperfusion and persistent or recurrent ischemia is therefore likely to occur in patients with ischemic heart disease.

We found that 7-day treatment with parecoxib reduced mortality, improved ventricular function, reduced LV cavity size, and reduced peri-infarct apoptosis in a mouse model of AMI due to permanent artery ligation (PI). These findings are consistent with several other studies in rats and mice.[4,6,9] On the other hand, we found no benefit or harm with parecoxib treatment in a mouse model of AMI due to transient ischemia with reperfusion (I/R). This second set of findings may conflict with prior studies in which COX-2 inhibition was associated with loss of protection in ischemic preconditioning.[2–3] Although additional studies would be necessary to determine how the different infarct models may influence COX-2 expression and/or activity, we believe that the current study illustrates how the choice of experimental model (and the duration of ischemia) can alter the response to pharmacologic intervention.

The beneficial effects of COX-2 inhibition on remodeling after PI are paralleled by a significant decrease in apoptosis, a key pathologic feature in post-infarction remodeling.[10–12] We speculate that the increased rate of peri-infarct apoptosis in mice with PI may be a consequence of increased COX-2 activity in the ischemic myocardium.[12] This may explain why parecoxib proved beneficial only in PI model and not in the I/R model.

We did not find any beneficial effects of parecoxib in the transient coronary occlusion model (I/R). The lack of residual or recurrent myocardial ischemia in this model may explain the absence of COX-2 stimulation in the peri-infarct myocardium and the lack of benefit from COX-2 inhibition. Alternatively, the difference may lay in the significantly smaller infarct size between the 2 models. Further studies are needed to look for effects of periods of ischemia longer than 30 minutes and/or longer follow-up and treatments in this model of transient

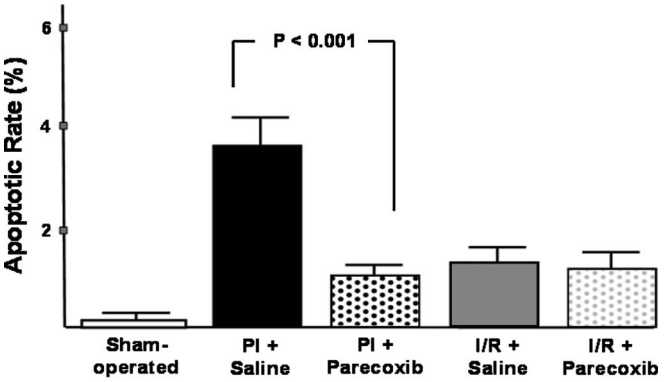

FIGURE 4. Effects of parecoxib on apoptosis in mice after PI or I/R.

ischemia. The discrepancy between 2 different models of myocardial ischemia highlights the importance of selecting appropriate preclinical models in translational research.

Several limitations of the study merit consideration. These include small sample size, short follow-up period, single-sex animal population, and the operator dependency of the TTE assessment of LV function. All investigators were blinded to treatment allocation during TTE to minimize the potential for operator bias. Although the lack of assessment of the area at risk in this study may be viewed as an additional potential limitation, we are confident that the areas at risk were comparable as the very same operator performed all procedures and the ligation site was similar in all cases independent of whether reperfusion was performed or not.

From a translational standpoint, most patients presenting with ST-segment elevation receive prompt and effective reperfusion that may mimic a model of transient I/R. However, the atherosclerosis and plaque rupture seen in acute coronary syndromes may be associated with more severe metabolic derangements than transient ischemia in otherwise healthy mice. Furthermore, suboptimal tissue level reperfusion (no-reflow phenomenon) occurs frequently in clinical practice and many patients receive no reperfusion at all, mostly due to late presentation.[13] Although limited in experimental design, these data suggest that patients with incomplete or no reperfusion may benefit from COX-2 inhibitors, whereas patients with short duration of ischemia and complete reperfusion may not. No single animal model of ischemic damage represents the optimal setting for all investigations. Investigators and clinicians should carefully evaluate the impact of different preclinical models when considering the translational potential of a novel intervention.

The translational potential of this study is hindered, however, by the concerns for parecoxib thrombogenicity and observations of increased adverse cardiovascular events in parecoxib patients undergoing cardiac surgery.[14–17] Although the mouse surgical ligation model may represent an adequate model to study the effects of myocardial ischemia, surgical induction of AMI in the mouse eliminates the possibility of studying the effects of parecoxib on those very factors that induce AMI in humans.

In conclusion, COX-2 inhibition by parecoxib inhibits apoptosis, ameliorates loss of cardiac performance, and improves survival in a mouse model of AMI due to permanent coronary occlusion but shows no benefit in a model of AMI due to transient ischemia.

ACKNOWLEDGMENT

The authors wish to thank Dr. Vera Di Trocchio-Abbate (Virginia Commonwealth University, Richmond, VA) for her editorial assistance.

REFERENCES

1. Shinmura K, Tang XL, Wang Y, et al. Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. *Proc Natl Acad Sci U S A*. 2000;97:10197–10202.
2. Bolli R, Shinmura K, Tang XL, et al. Discovery of a new function of cyclooxygenase (COX)-2: COX-2 is a cardioprotective protein that alleviates ischemia/reperfusion injury and mediates the late phase of preconditioning. *Cardiovasc Res*. 2002;55:506–519.
3. Saito T, Rodger IW, Hu F, et al. Inhibition of COX pathway in experimental myocardial infarction. *J Mol Cell Cardiol*. 2004;37:71–77.
4. LaPointe MC, Mendez M, Leung A, et al. Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. *Am J Physiol Heart Circ Physiol*. 2004;286:H1416–H1424.
5. Abbate A, Limana F, Capogrossi MC, et al. Cyclo-oxygenase-2 (COX-2) inhibition reduces apoptosis in acute myocardial infarction. *Apoptosis*. 2006;11:1061–1063.
6. Straino S, Salloum FN, Baldi A, et al. Protective effects of parecoxib, a cyclo-oxygenase-2 inhibitor, in postinfarction remodeling in the rat. *J Cardiovasc Pharmacol*. 2007;50:571–577.
7. Sivarajah A, McDonald MC, Thiemermann C. The cardioprotective effects of preconditioning with endotoxin, but not ischemia, are abolished by a peroxisome proliferator-activated receptor-gamma antagonist. *J Pharmacol Exp Ther*. 2005;313:896–901.
8. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr*. 1989;2:358–367.
9. Abbate A, Salloum FN, Ockaili RA, et al. Improvement of cardiac function with parecoxib, a cyclo-oxygenase-2 inhibitor, in a rat model of ischemic heart failure. *J Cardiovasc Pharmacol*. 2007;49:416–418.
10. MacLellan WR, Schenider MD. Death by design. Programmed cell death in cardiovascular biology and disease. *Circ Res*. 1997;81:137–144.
11. Abbate A, Bussani R, Biondi-Zoccai GGL, et al. Infarct-related artery occlusion, tissue markers of ischaemia and increased apoptosis in the peri-infarct viable myocardium. *Eur Heart J*. 2005;26:2039–2045.
12. Sam F, Sawyer DB, Chang DL, et al. Progressive left ventricular remodeling and apoptosis late after myocardial infarction in mouse heart. *Am J Physiol Heart Circ Physiol*. 2000;279:H422–H428.
13. Abbate A, Kontos MC, Biondi-Zoccai GGL. No re-flow: the next challenge in treatment of ST-elevation acute myocardial infarction. *Eur Heart J*. 2008;29:1795–1797.
14. Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest*. 2006;116:4–15.
15. Buerkle MA, Lehrer S, Sohn HY, et al. Selective inhibition of cyclooxygenase-2 enhances platelet adhesion in hamster arterioles in vivo. *Circulation*. 2004;110:2053–2059.
16. Nussmeier NA, Whelton AA, Brown MT, et al. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N Engl J Med*. 2005;352:1081–1091.
17. Ott E, Nussmeier NA, Duke PC, et al. Efficacy and safety of the cyclooxygenase 2 inhibitors parecoxib and valdecoxib in patients undergoing coronary artery bypass surgery. *J Thorac Cardiovasc Surg*. 2003;125:1481–1492.