Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse

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Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. Am J Physiol Heart Circ Physiol 286: H1416–H1424, 2004; 10.1152/ajpheart.00136.2003.—Cyclooxygenase (COX)-2 is expressed in the heart in animal models of ischemic injury. Recent studies have suggested that COX-2 products are involved in inflammatory cell infiltration and fibroblast proliferation in the heart. Using a mouse model, we questioned whether 1) myocardial infarction (MI) in vivo induces COX-2 expression chronically, and 2) COX-2 inhibition reduces collagen content and improves cardiac function in mice with MI. MI was produced by ligation of the left anterior descending coronary artery in mice. Two days later, mice were treated with 3 mg/kg NS-398, a selective COX-2 inhibitor, or vehicle in drinking water for 2 wk. After the treatment period, mice were subjected to two-dimensional M-mode echocardiography to determine cardiac function. Hearts were then analyzed for determination of infarct size, interstitial collagen content, brain natriuretic peptide (BNP) mRNA, myocyte cross-sectional area, and immunohistochemical staining for transforming growth factor (TGF)-β and COX-2. COX-2 protein, detected by immunohistochemistry, was increased in MI versus sham hearts. MI resulted in increased left ventricular systolic and diastolic dimension and decreased ejection fraction, fractional shortening, and cardiac output. NS-398 treatment partly reversed these detrimental changes. Myocyte cross-sectional area, a measure of hypertrophy, was decreased by 30% in the NS-398 versus vehicle group, but there was no effect on BNP mRNA. The interstitial collagen fraction increased from 5.4 ± 0.4% in sham hearts to 10.4 ± 0.9% in MI hearts and was decreased to 7.9 ± 0.6% in NS-398-treated hearts. A second COX-2 inhibitor, rofecoxib (MK-0966), also decreased myocyte cross-sectional area and interstitial collagen fraction. TGF-β, a key regulator of collagen synthesis, was increased in MI hearts. NS-398 treatment reduced TGF-β immunostaining by 40%. NS-398 treatment had no effect on BNP mRNA. The interstitial collagen fraction increased by 10.2±0.3% on October 14, 2017 http://ajpheart.physiology.org/ Downloaded from

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Cyclooxygenase (COX), also known as prostaglandin endoperoxide H synthase, exists as two isoforms, COX-1 and COX-2 (43). COX-1 is expressed in almost all tissues, and its prostanooid products are thought to mediate physiological responses such as vascular homeostasis and protection of the gastric mucosa. Although COX-2 products are generally considered inflammatory mediators and thus deleterious, they can exhibit both beneficial and deleterious effects in the same tissue, for example, the kidney (14, 44). In addition to inflammation, COX-2 products have been associated with regulation of cell proliferation, in particular colon cancer (12, 45). Prostanoids produced by COX-2 affect a number of other biological processes, including angiogenesis, differentiation, and apoptosis (24, 46). HEK-293 cells overexpressing both COX-2 and PGE2 synthase release large quantities of PGE2, grow faster than normal cells, and also exhibit altered morphology (30). In addition, COX-2 has been detected in the heart in patients with congestive heart failure and during allograft rejection (47, 50). However, we know very little about its regulation in the heart or its contribution to cardiac structure and function.

COX-1 and -2 catalyze conversion of arachidonic acid to the prostaglandin endoperoxide PGH2 by similar mechanisms. Arachidonic acid released by proinflammatory stimuli tends to be preferentially utilized by COX-2, and when COX-2 and COX-1 are functional in the same cell, COX-2 can form up to four times as much PGH2 as COX-1 (40). PGH2 is converted to a variety of prostanooids including PGE2, PGI2, PGF2α, PGI2, and thromboxane A2 by specific synthases (12, 46), and a specific synthase seems to be functionally coupled to either COX-1 or -2 within the cell to produce a particular prostanooid. For example, COX-2 and inducible PGE2 synthase (PGES) are coupled to generate PGE2 in the inflammatory response (30). We have previously shown that the cytokine IL-1β induces both COX-2 and PGES in neonatal ventricular myocytes and that PGE2 and PGF2α, but not a PGI2 analog, stimulate hypertrophic growth of myocytes (27).

In contrast to hormones, which have broad systemic effects, prostanooids serve as intracrine, autocrine, and paracrine mediators, signaling changes within the immediate environment. Uregulation of COX in the heart could produce prostanooid products that would affect cell function. Previous studies examining the role of COX in the heart used nongonselective COX inhibitors, and these studies produced variable results (4, 6, 18, 29). In recent studies in rodents, COX-2 selective inhibitors were given before myocardial infarction (MI) and resulted in decreased fibroblast proliferation and improved cardiac function (36, 38). However, the studies did not distinguish between the effects of COX-2 inhibitors on wound healing and scar formation as well as subsequent remodeling. In an attempt to clarify some of the processes regulated by COX-2 in the heart, we studied the effects of administering a COX-2 inhibitor 48 h post-MI and for an additional 2 wk on cardiac function, fibrosis, and hypertrophy.

METHODS

Animal Procedures

Male C57BL/6J mice from Jackson Laboratories were housed in a temperature-controlled room with a 12:12-h light-dark cycle and given standard chow and tap water. They were housed for at least 1 wk before the start of the experiment, which was approved by the
Experimental Protocols

**Protocol 1: effect of NS-398 on cardiac PGE₂.** Mice were subjected to MI to document an increase in cardiac COX-2 and PGE₂ and to test the ability of NS-398 (NS) to inhibit COX-2-derived PGE₂. After 24 h, the LV was divided into infarcted and noninfarcted regions, and RNA was isolated for real-time RT-PCR analysis of COX-2 mRNA. A second group of mice was subjected to MI, and, after 24 h, hearts were extracted for determination of PGE₂ levels. To insure that NS inhibited MI-induced PGE₂, a third group of MI mice was treated three times daily with NS (on the day before surgery, 2 h before surgery, and 2 h before death). Twenty-four hours after surgery, hearts were removed and extracted for PGE₂ analysis.

Hearts were homogenized in 1 ml of methanol containing 10 μg/ml indomethacin. The volume of methanol was adjusted to 4 ml, and PGE₂ was extracted by repeated vortexing over a 30-min period. After centrifugation at 4,500 rpm at 4°C, the supernatant was purified through PGE₂ affinity columns according to the manufacturer’s instructions (Cayman Chemicals; Ann Arbor, MI). After the sample was eluted from the column, the sample was dried in a Savant and then resuspended in 0.4 ml assay buffer. One-half of the sample was frozen, whereas the other half was analyzed for PGE₂ with an enzyme-linked immunoassay kit from Cayman Chemicals according to the manufacturer’s protocol. Results were recorded as picograms per sample. PGE₂ (200 pg) was added to an affinity column to measure recovery. Recovery of PGE₂ from the columns averaged >95%.

**Protocol 2: effect of NS on cardiac structure and function.** Mice with MI were divided into two groups and started on 3 mg·kg⁻¹·day⁻¹ NS (Cayman Chemicals) or vehicle in drinking water 48 h after MI and lasting for 2 wk. Sham-operated mice received tap water. In the first 5–6 days of treatment, there was no apparent difference in survival of vehicle-treated and NS-treated mice.

After the treatment period, mice were subjected to two-dimensional M-mode echocardiography to determine cardiac function, and mice were then euthanized. Hearts were removed and sectioned transversely into four slices from apex to base. Three of the sections (sections A, B, and D) were frozen in isopentane and stored at −70°C for determination of infarct size (IS), interstitial collagen fraction (ICF), myocardocyte cross-sectional area (MCSA), and immunohistochemical staining for transforming growth factor (TGF)-β and COX-2. The fourth section (section C) was stored in RNALater (Ambion) at −70°C for subsequent extraction of RNA. Section A contained mostly infarcted tissue, and section B was adjacent to the infarct, whereas sections C and D contained noninfarcted myocardium.

**Protocol 3: effect of rofecoxib on MCSA and ICF.** To verify the histopathology studies described in protocol 2, we tested a second COX-2 inhibitor, rofecoxib (MK-0966; kindly provided by Dr. Robert Young, Merck Frosst, Quebec, Canada). The drug was administered in chow [0.0075% rofecoxib (w/w)] in chow = 15 mg·kg⁻¹·day⁻¹] beginning at MI and continuing for 2 wk. RNA was isolated from 11 mice fed this dose for 2 wk gave rofecoxib values of 109 ± 16 ng/ml, consistent with therapeutic levels in humans (values kindly determined by Pauline Luk, Merck Frosst, Quebec, Canada).

**Echocardiography**

Two-dimensional M-mode transthoracic echocardiography was performed on conscious mice using an Acuson 256 system (Mountain View, CA) with a 15-MHz linear transducer as reported previously (15). M-mode images were used to determine LV end-diastolic dimension (LVDd) and LV end-systolic dimension (LVDs). Diastolic measurements were made at the maximum LV cavity dimension, whereas systolic parameters were measured during maximum anterior motion of the posterior wall.

**Infarct Size**

IS was measured as described previously (51). For each heart, 6-μm sections from each of the three slices of sections A, B, and D were stained with Gomori trichome to identify fibrous tissue (infarction). IS was calculated as the ratio of infarct length to the circumference of both the endocardium and epicardium.

**Measurement of ICF and MCSA**

Slices from sections A, B, and D of the heart were double stained with fluorescein-labeled peanut agglutinin to delineate the MCSA and interstitial space and rhodamine-labeled *Griffonia simplicifolia* lectin I to outline the capillaries (22). Four radially oriented microscope fields were selected from each section, two adjacent to the infarct border zone and two away from it, and photographed at a magnification of ×100. MCSA was measured by computer-based planimetry (Jandel) and averaged across all four fields of the sections from the three slices. To determine ICF, the total surface area (microscope field), interstitial space (collagen plus capillaries), and the area occupied by capillaries alone were measured with videodensitometry (SigmaScan, Jandel). ICF was calculated as the total surface area occupied by the interstitial space minus the percentage of total surface area occupied by the capillaries.

**Detection of COX-2 and TGF-β by Immunostaining**

Frozen sections (6 μm) of section B of the heart were fixed with acetone for 10 min and rinsed in PBS. Tissue sections were preincubated with 0.3% hydrogen peroxide in PBS to inhibit endogenous peroxidase activity. They were then washed twice in PBS for 5 min each time, preincubated with blocking serum (5% normal serum) for 30 min, and then incubated with the primary antibody at 4°C overnight. For COX-2 immunostaining, sections were incubated with a 1:500 dilution of COX-2 antibody (M-19, Santa Cruz Biotechnology). For detection of TGF-β, a 1:500 dilution of monoclonal antibody was used (MAB 1835, R&D Systems). After sections were washed with PBS, species-appropriate biotinylated secondary antibodies were applied, followed by avidin-peroxidase complexes (Vector Laboratories). 3-Amino-9-ethyl carbazole was used as the substrate (Vector Laboratories), and slides were counterstained with Gill’s hematoxylin solution. For quantitation of TGF-β staining, two fields enclosing the infarct border were photographed using a Nikon microscope attached to a digital camera. The image was obtained using SPOT software (version 3.4.2, Diagnostic Instruments) at ×400 magnification. The area that stained positive for TGF-β was expressed as a percentage of the total myocardial area. All slides were evaluated in blinded fashion.

**Analysis of Brain Natriuretic Peptide and COX-2 mRNA by Real-Time PCR**

Total RNA was extracted by homogenization of myocardial samples in Tri-Reagent (Molecular Research Center, Cincinnati, OH) following the manufacturer’s instructions. For brain natriuretic peptide (BNP) mRNA, the section of the mouse heart homogenized included a portion of the noninfarcted LV free wall and the septum (section C from protocol 2). For COX-2 mRNA, the LV was divided into infarcted and noninfarcted regions, which were extracted sepa-
rately. At the end of the procedure, samples were treated with DNAse I from Ambion’s RNasequeous-4PCR kit. RT was performed using the reagents of the Omniscript RT kit (Qiagen) with 2 μg total RNA, 1 μg random primer (Invitrogen), and 10 μl RNAsin (Promega) in a 20-μl volume for 1 h at 37°C.

Real-time PCR was performed using the QuantiTect Probe PCR kit (Qiagen) and a Roche LightCycler (version 3.5). Fluorescence resonance energy transfer probes and primers were designed by TIB MolBiol (Adelphia, NJ). Data analysis was performed with LightCycler software (version 3.5.28). The reaction volume was 20 μl and contained 2 μl cDNA, 0.5 μM sense and antisense primers, 0.2 μM each of the LC-640 red-labeled and fluorescein-labeled probes, Taq polymerase, and dNTPs for 45 cycles. For analysis of BNP (241 bp) and β-actin (342 bp), PCR conditions were as follows: 0 s at 95°C for denaturation, 40 s at 58°C for annealing, and 40 s at 72°C for extension. For COX-2 (448 bp), PCR conditions were as follows: 0 s at 95°C for denaturation, 45 s at 58°C for annealing, and 40 s at 72°C for extension. The sequences of the primers/probes were as follows: BNP sense, 5’-ATGGATCTCCTGAGGTTGCTG-3’; BNP antisense, 5’TGCTGCTGCTGAGACCAGAA-3’; BNP fluorescein probe, 5’TGGCTAGCATCCAGAGGATAAG-3’; BNP LC-640 probe, 5’-LC red 640-TGACCTCCAGGCTGAGCATATAAA-p-3’; β-actin sense, 5’-ACCCACACTGTTGCCCCATCTA-3’; β-actin antisense, 5’-GCCACAGATTTCCATACACCA-3’; β-actin fluorescein probe, 5’-GCCACGCTGGTCAGATCTTCTAT-x-3’; β-actin LC-705 probe, 5’-LC red 705-AGGTAGTCTGTCAGGTCCCGGCCA-p-3’; COX-2 sense, 5’-GTGACTGTACCCGGACTGGAT-3’; COX-2 fluorescein probe, 5’-GTGACTGTACCCGGACTGGAT-3’; COX-2 LC-640 probe, 5’-CTGGAGGACGCTGCTCCAGATGC-3’; COX-2 fluorescein probe, 5’-CTGGAGGACGCTGCTCCAGATGC-3’; and COX-2 LC-640 probe, 5’-ACTACACAGGGCCCTTCCTCCC-p-3’. Gene-specific standard curves were generated using a linearized plasmid containing the cDNA of interest and serially diluting it to generate concentrations ranging over two to four orders of magnitude. The LightCycler software calculated the amount of cDNA in a given sample (copies/μl) compared with the standard curve. BNP and COX-2 mRNA were normalized to β-actin mRNA.

Statistical Analysis

Data were tested for normality and equality of variance and adjusted appropriately. If the data were not normally distributed, then the nonparametric Wilcoxon rank-sum method was used. The Satterthwaite adjustment was used for data with unequal variances. If the data were not normally distributed, then the Wilcoxon rank-sum method was used. The Satterthwaite adjustment was used for data with unequal variances. BNP mRNA data were analyzed by the nonparametric Wilcoxon rank-sum method. All other data are expressed as means ± SE and were analyzed by r-test or one-way ANOVA, with multiple pairwise comparisons made by the Student-Newman-Keuls method. TGF-β data were analyzed by r-test with the Satterthwaite adjustment for unequal variances. P < 0.05 was considered significant.

**RESULTS**

**Effect of MI on COX-2 and PGE2 in the Heart**

To test the acute effect of MI on COX-2 mRNA and PGE2 levels in the heart, we analyzed total RNA from the infarcted and noninfarcted regions of the LV free wall 24 h post-MI. COX-2 mRNA normalized to β-actin mRNA was increased 4.4-fold in the infarcted LV (Fig. 1A). Cardiac PGE2 levels were increased 3.7-fold after MI (Fig. 1B).

Doses of NS ranging from 1 to 10 mg·kg⁻¹·day⁻¹ have been shown to be efficacious in several different models (13, 25, 37, 39). PGE2 in sham hearts was 6.9 pg/sample (n = 8), which increased to 26.52 pg/sample (n = 12) in MI hearts. Treatment of mice with 3 mg·kg⁻¹·day⁻¹ NS reduced cardiac PGE2 levels to 12.5 pg/sample (n = 9) (Fig. 1B). As a control for the PGE2 assay, we used indomethacin (10 mg/kg), a COX-1/COX-2 inhibitor, and found that PGE2 was decreased to levels below the lower limit of detection of the assay.

To study chronic induction of COX-2 in MI hearts, we stained sections of hearts from a separate group of animals for COX-2 immunoreactivity. Part of section B (adjacent to the infarct) from sham and MI hearts was immunostained using a polyclonal COX-2 antibody. Sections from sham hearts showed no staining, but sections from the heart 16 days post-MI showed very intense localized brown staining in the myocytes (Fig. 2).

**Effect of COX-2 Inhibition on Cardiac Function**

We used two-dimensional M-mode echocardiography to evaluate cardiac function in untreated and NS-treated mice. Ejection fraction, shortening fraction, stroke volume, and cardiac output decreased in MI versus sham mice (Fig. 3). In all cases, COX-2 inhibition partly reversed the detrimental effects of MI. COX-2 inhibition had no effect on heart rate (data not shown). MI also increased both LVDs and LVDd (Fig. 4, A and B). COX-2 inhibition significantly reduced LVDs (P < 0.01) and tended to reduce LVd (P = 0.09, NS-treated MI mice versus vehicle-treated MI mice).

**Effect of COX-2 Inhibition on Hypertrophy and Fibrosis**

Using the heart sections, we next measured MCSA as an index of hypertrophy. MCSA increased 2-fold in MI versus sham hearts, and COX-2 inhibition reduced this to 1.7-fold (sham: 149 μm²; MI: 302 μm²; MI + NS: 257 μm²; P < 0.05, MI vs. NS; Fig. 5A). Because increases in the expression of
atrial natriuretic peptide and BNP genes are markers of hypertrophy, we used real-time PCR to quantitate changes in BNP gene expression in sham, MI, and NS-treated mouse hearts. There was a 3.6-fold increase in BNP mRNA in MI versus sham hearts, but COX-2 inhibition had no significant effect on BNP mRNA (Fig. 5B).

We measured changes in ICF in sections from sham, MI, and MI + NS hearts as a measure of collagen in noninfarcted areas of the heart. The ICF of sham hearts was 5.4% and increased twofold to 10.4% in MI hearts (Fig. 6). In NS-treated hearts, ICF was decreased by 50% to 7.9%, indicating decreased collagen production ($P < 0.05$, MI vs. NS treated).

To confirm the MCSA and ICF data, studies were repeated using a second COX-2 inhibitor, rofecoxib (MK-0966 or Vioxx). Treatment with rofecoxib for 2 wk decreased MCSA (Fig. 7A) and ICF (Fig. 7B) by 65% and 48%, respectively. Additionally, if mice were treated for 4 wk beginning 2 wk after MI, MCSA and ICF were similarly decreased (Fig. 7, C and D).

**Effect of COX-2 Inhibition on TGF-β**

After MI, there is a sequential infiltration of the heart by inflammatory cells, such as neutrophils, macrophages, and lymphocytes (49), which contribute to removal of necrotic tissue, scar formation, and interstitial fibrosis. TGF-β, which is synthesized and released by macrophages and fibroblasts, results in fibroblast proliferation and collagen synthesis (for a review, see Ref. 23). We examined slices of section B of the heart (adjacent to the scar) for TGF-β (Fig. 8A) and quantified the immunostained area as a percentage of the entire section examined. TGF-β staining increased from 0.4% in sham hearts...
to 1.2% in MI hearts and decreased by 50% to 0.7% in response to COX-2 inhibition by NS (Fig. 8B).

**Effect of COX-2 Inhibition on IS**

To test whether the cardioprotective effects of COX-2 inhibition were due to a decrease in IS, we measured IS in tissue sections from MI hearts treated with vehicle and MI hearts treated with NS. We found that IS as a percentage of the entire LV was unchanged by COX-2 inhibition [IS in the MI/vehicle group = 40 ± 3% (n = 9) and in the MI/NS group = 37 ± 4% (n = 8)]. The same was true for rofecoxib treatment [IS in the MI/vehicle group = 37.1 ± 1.5% (n = 12) and in the MI/rofecoxib group = 37.8 ± 1.6% (n = 12)].

**DISCUSSION**

As an early inflammatory response gene, COX-2 is induced by a variety of stimuli. In the myocardium it is stimulated by cardiac allograft rejection (50) and MI (38) as well as during the development of heart failure (47). Our data show that 1) MI induced COX-2 in the mouse heart, 2) COX-2 inhibition with NS improved cardiac function, and 3) both NS and rofecoxib reduced MCSA and interstitial collagen. These data thus implicate COX-2 products in cardiac dysfunction after MI. Induction of COX-2 by injury has also been reported in other tissues. COX-2 expression was induced in the brain in different models of injury (11, 17, 19, 31), and inhibition of COX-2 by NS or gene knockout improved function after injury (17, 31).

In mice, LAD ligation causes ischemia of the LV free wall and necrosis of infarcted tissue, followed by scar formation and remodeling of the noninfarcted myocardium. In this mouse model, LAD ligation results in rapid increases in LVDs and LVDD and decreased ejection fraction and cardiac output (51). We found that COX-2 inhibition with NS improved cardiac function, as evidenced by increased ejection fraction, shortening fraction, cardiac output, and stroke volume versus untreated mice. It is likely that the increases in ejection fraction and shortening fraction were due to improved cardiac contractility, because LVDD was not changed, whereas LVDs decreased significantly. Two other reports have focused on the role of COX-2 in the heart post-MI, but in both studies the COX-2 inhibitor was given before MI. Scheuren et al. (38) treated female rats with rofecoxib one day before MI and then for 4 days afterward. Their acute studies revealed decreased infiltration of inflammatory cells and decreased fibroblast proliferation and invasion of the infarct border. No functional studies were done, and no conclusions were drawn concerning the potential beneficial or deleterious effects of the acute reduction in inflammatory processes. In contrast, Saito et al. (36) administered the COX-2 selective inhibitor 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2 (5H)-furanone to male rats 30 min before MI and continued treatment for 2 wk. After another 2 wk, hemodynamic studies indicated that COX-2 inhibition reduced LV end-diastolic pressure and improved cardiac compliance, resulting in an overall increase in cardiac function. This supports our finding of an improvement in LVDs subsequent to NS treatment.

Our studies also indicate that COX-2 inhibition reduced collagen deposition in the heart after MI. This beneficial alteration in the remodeling process probably reduced cardiac stiffness, thus accounting for the improved cardiac function. The decrease in collagen likely resulted from decreased TGF-β release by either macrophages, myocytes, or fibroblasts, as we detected less TGF-β immunostaining in NS-treated hearts. Our result is consistent with reports using two different models of renal injury in the rat. In these studies, chronic COX-2 inhibition reduced the extracellular matrix, TGF-β production, glomerular injury, and proteinuria, thus improving renal function (8, 44).
The decrease in collagen content in our study most likely resulted from decreased proliferation of fibroblasts and decreased infiltration of inflammatory cells, which synthesize and release cytokines and growth factors involved in extracellular matrix formation. This was demonstrated by Scheuren et al. (38), who noted decreased numbers of macrophages and fibroblasts around the infarct area of rofecoxib-treated rats. Preliminary data from our laboratory suggest that the PGE2 analog sulprostone and PGF2α increase fibroblast proliferation in vitro (M. Mendez and M. C. LaPointe, unpublished observations). In addition to affecting fibroblast proliferation, prostanoids could participate in the acute inflammatory response by upregulation of the adhesion molecules necessary for infiltration and activation of neutrophils. The prostanoid PGF_{2α} has been shown to stimulate ICAM-1 expression in gingival fibroblasts (32). COX-2 inhibitors have also been shown to decrease plasma levels of lipid peroxides, reduce cytokine release and/or action, and decrease production of superoxide anions by neutrophils (2, 3, 41). Thus the effect of COX-2 inhibition in reducing collagen production could result from a combination of direct effects on cardiac prostanoid formation, which would influence neutrophil and macrophage infiltration, fibroblast proliferation, and TGF-β and collagen synthesis.

COX-2 inhibition also reduced cardiac hypertrophy, as determined by measurements of MCSA. It has been reported that the nonsteroidal anti-inflammatory drug fenbufen prevented cardiac hypertrophy induced by clenbuterol in rats (35). Enhanced COX-2 expression in the heart would increase prostanoid production. We have previously shown that PGE2 acting through the EP receptor subtype EP4 stimulates hypertrophy of cardiac myocytes in vitro (27). The presence of EP4 receptors in the heart would allow locally produced PGE2 to induce hypertrophy of myocytes. Other prostanoids, including PGF_{2α} and thromboxane A2, can also stimulate hypertrophy (1, 20, 27) as well as stimulate BNP expression (M. C. LaPointe, unpublished observations).

BNP mRNA, a marker of hypertrophy and LV dysfunction, was induced by MI, but its levels were unchanged by COX-2...
inhibition. The uncoupling of BNP expression from regression of hypertrophy has been previously reported. Using a model of suprarenal aortic banding, Ogawa et al. (33) demonstrated that when both hypertrophy and blood pressure were decreased by high-dose ramipril (an angiotensin-converting enzyme inhibitor), LV BNP mRNA was reduced. However, when hypertrophy was regressed without decreased blood pressure by low-dose ramipril, LV BNP mRNA remained elevated. Given that BNP decreases collagen synthesis and increases matrix metalloproteinase in fibroblasts in vitro (42), its continued synthesis in vivo might counter collagen accumulation in the setting of COX-2 inhibition.

We found that COX-2 inhibition with either NS or rofecoxib (MK-0966) beginning 2 days post-MI had no effect on IS measured 14 days later. Scheuren et al. (38) also found that rofecoxib did not influence IS acutely in the rat. Although we did not detect any changes in overall IS in our study, we cannot exclude the possibility that COX-2 inhibition affected wound repair by altering infarct expansion or infarct thinning. A decrease in collagen in the scar per se could predispose the heart to rupture. Future studies will examine COX-2 inhibition in the chronic phases of remodeling so as to avoid potential effects on wound healing.

There is some controversy regarding the role of COX-2 in the heart post-MI. Recent studies have indicated that rofecoxib may increase the risk of cardiovascular events in patients (5, 28). Cheng et al. (9) demonstrated that the vasodilator PGI2 is critical to oppose the actions of platelet thromboxane A2, and McAdam et al. (26) indicated that COX-2 is important for systemic synthesis of PGI2. If COX-2 is important for production of PGI2 in the vessel wall, then COX-2 inhibition might favor thrombosis. On the other hand, several studies have implicated COX-2 and its products in cardiovascular disease. Chenevard et al. (7) found that the COX-2 inhibitor celecoxib improved flow-mediated dilatation and decreased markers of inflammation and oxidative stress in male patients with severe coronary artery disease. COX-2 overexpression has also been associated with atherosclerotic plaque instability, which would lead to acute ischemic syndromes (10). Moreover, COX-2 expression is strongly induced during LPS-induced sepsis in rats, and treatment with the COX-2 inhibitor rofecoxib reversed the effects of LPS on systolic arterial pressure and heart rate as well as liver damage (16). It has also been shown that COX-2 is involved in the inflammatory process after MI. COX-2 inhibition reduced macrophage infiltration into the necrotic area and border zone of the infarcted rat heart as well as fibroblast proliferation (38, 48). Thus additional studies will be required to evaluate whether COX-2 inhibitors as a class have prothrombotic properties under certain circumstances, or if there are specific properties of the different COX-2 inhibitors that render them prothrombotic, as well as determine the different effects of COX-2 and prostanoid production on myocyte, fibroblast, and endothelial cell function. Because different cell types in the heart, e.g., myocytes, fibroblasts, and endothelial cells, produce different prostanoid profiles (21, 27, 34), the effect of induction of COX-2 will depend on the cell in which it is expressed, the prostanoids produced, and the ability of surrounding cells to respond to the prostanoids.

In summary, our findings suggest that COX-2 plays a deleterious role in the heart after MI caused by chronic LAD occlusion. MI induces COX-2, with concomitant increases in PGE2, collagen content, and hypertrophy, and decreases in
cardiac function. COX-2 inhibition partly reverses these effects. Thus selective and timely use of COX-2 inhibitors may improve cardiac function after ischemic injury.

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