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# Cyclooxygenase-1 and -2-Dependent Prostacyclin Formation in Patients With Atherosclerosis

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**Background**—The formation of prostacyclin (PGI<sub>2</sub>), thromboxane (TX) A<sub>2</sub>, and isoprostanes is markedly enhanced in atherosclerosis. We examined the relative contribution of cyclooxygenase (COX)-1 and -2 to the generation of these eicosanoids in patients with atherosclerosis.

**Methods and Results**—The study population consisted of 42 patients with atherosclerosis who were undergoing surgical revascularization. COX-2 mRNA was detected in areas of atherosclerosis but not in normal blood vessel walls, and there was evidence of COX-1 induction. The use of immunohistochemical studies localized the COX-2 to proliferating vascular smooth muscle cells and macrophages. Twenty-four patients who did not previously receive aspirin were randomized to receive either no treatment or nimesulide at 24 hours before surgery and then for 3 days. Eighteen patients who were receiving aspirin were continued on a protocol of either aspirin alone or a combination of aspirin and nimesulide. Urinary levels of 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-6-keto-PGF<sub>1α</sub>, metabolites of TXA<sub>2</sub> and PGI<sub>2</sub>, respectively, were elevated in patients with atherosclerosis compared with normal subjects (3211±533 versus 679±63 pg/mg creatinine, *P*<0.001; 594±156 versus 130±22 pg/mg creatinine, *P*<0.05, respectively), as was the level of the isoprostane 8-iso-PGF<sub>2α</sub>. Nimesulide reduced 2,3-dinor-6-keto-PGF<sub>1α</sub> excretion by 46±5% (378.3±103 to 167±37 pg/mg creatinine, *P*<0.01) preoperatively and blunted the increase after surgery. Nimesulide had no significant effect on 11-dehydro-TXB<sub>2</sub> before (2678±694 to 2110±282 pg/mg creatinine) or after surgery. The levels of both products were lower in patients who were taking aspirin, and no further reduction was seen with the addition of nimesulide. None of the treatments influenced urinary 8-iso-PGF<sub>2α</sub> excretion.

**Conclusions**—Both COX-1 and -2 are expressed and contribute to the increase in PGI<sub>2</sub> in patients with atherosclerosis, whereas TXA<sub>2</sub> is generated by COX-1. (*Circulation*. 2000;102:840-845.)

**Key Words:** atherosclerosis ■ cyclooxygenase ■ prostaglandins

Atherosclerosis is an inflammatory lesion that is characterized by mononuclear infiltration and smooth muscle cell proliferation.<sup>1-4</sup> As with inflammation at other sites, atherosclerosis is associated with an increase in prostaglandin biosynthesis.<sup>5</sup> The products include thromboxane (TX)A<sub>2</sub>, a potent platelet activator, vasoconstrictor, and smooth muscle mitogen,<sup>6</sup> and prostacyclin (PGI<sub>2</sub>), a platelet inhibitor and vasodilator.<sup>7</sup> TXA<sub>2</sub> is in large part generated by platelets in normal subjects, but it may also be formed by nucleated cells such as monocytes.<sup>8-10</sup> PGI<sub>2</sub> is generated by large vessel endothelium and vascular smooth muscle cells (VSMCs).<sup>11</sup> In addition to prostaglandins, there is an increased formation of isoprostanes, which are isomers of prostaglandins formed by free radical oxidation of arachidonic acid.<sup>12,13</sup> Some isoprostanes, such as 8-iso-PGF<sub>2α</sub> can also be formed by cyclooxygenase (COX).<sup>14,15</sup> Isoprostanes are of interest not only as markers of oxidant injury but also as physiological mimics of prostaglandins.<sup>16,17</sup> For example, 8-iso-PGF<sub>2α</sub> activates platelets and VSMCs in a manner similar to TXA<sub>2</sub>. Thus, several

products are generated in atherosclerosis that may influence the development of the disease or the risk of thrombosis.

Prostaglandins and TXA<sub>2</sub> are synthesized from arachidonic acid by the enzyme COX. There are 2 isoforms of this enzyme that are the products of distinct genes.<sup>18,19</sup> COX-1 is constitutively expressed in most tissues and is the only functioning COX in platelets. COX-2 is an inducible form of the enzyme and is barely detectable in most tissues under normal physiological conditions.<sup>20</sup> However, recent studies demonstrate that COX-2 is a major source of PGI<sub>2</sub> in normal subjects.<sup>21-23</sup> COX-2 expression is increased by free radicals,<sup>24</sup> cytokines,<sup>25</sup> growth factors,<sup>26</sup> hormones,<sup>27</sup> and hypoxia,<sup>28</sup> stimuli that are implicated in the development of atherosclerosis.<sup>1</sup> Consequently, COX-2 may be responsible for the increase in prostaglandin formation seen in this condition. There is evidence that cytokines also induce the expression of COX-1, which has been implicated as a source of prostaglandins at sites of inflammation.<sup>29,30</sup> We examined the expression of COX isoforms in human atherosclerotic plaque and the effect of nimesulide, a selective COX-2 inhibitor,<sup>21</sup> on prostaglandin formation in patients with atherosclerosis.

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## Methods

### Subjects

The study was approved by the Irish Medicines Board and the Ethics Committee of Beaumont Hospital Dublin. All patients gave written informed consent. Forty-two patients (mean age  $70 \pm 2$  years, 36 men and 6 women) with clinical and angiographic evidence of atherosclerosis were studied. Eighteen patients were smokers, 19 were ex-smokers, and 5 were nonsmokers. Twenty-one patients were hypertensive, and 10 had a history of ischemic heart disease. Four patients had diabetes mellitus, and 3 patients were being treated for hypercholesterolemia. All patients were undergoing surgical revascularization for peripheral vascular disease or carotid endarterectomy. The study was open label and nonblinded. Patients ( $n=24$ ) who were not previously taking aspirin or any other nonsteroidal anti-inflammatory drug were randomized to receive either no treatment or 100 mg nimesulide BID beginning 24 hours before surgery and continuing for 3 days. Patients already taking aspirin ( $n=18$ ) were randomly assigned to continue to take 300 mg/d aspirin alone or a combination of 300 mg/d aspirin and 100 mg nimesulide BID. This relatively high dose of aspirin was selected because the study was conducted for only 3 days and because optimal inhibition of platelet COX may be delayed with low-dose aspirin regimens.<sup>8</sup> Patients with a history of active peptic ulcer disease or with renal or hepatic impairment were excluded from the study. Blood for serum TXB<sub>2</sub> and endotoxin-induced PGE<sub>2</sub> (COX-1 and -2 bioactivity, respectively) levels was collected at baseline and 1 hour after the first dose of drug. Urine was collected during each 24-hour period for the determination of prostaglandin metabolite and isoprostanol excretion. Samples of femoral or aortic tissue were obtained at the time of surgery for COX expression analysis. Samples were either fixed immediately in formaldehyde for immunohistochemistry or treated with Tri-Reagent (Sigma) for RNA extraction.

### Reverse Transcription-Polymerase Chain Reaction for COX Isoforms in Atherosclerotic Tissue

COX-1, COX-2, and GAPDH mRNA were extracted and detected with reverse transcription-polymerase chain reaction as described previously.<sup>24,31</sup> Each primer pair was designed to span at least 1 intron of the gene. The primers used were COX-2, 5'-TCAAA-TGAGATTGTGGGAAAATTG-3' (sense), 5'-TCTAGTAGAGAC-GGACTCATAGAA-3' (antisense); COX-1, 5'-TGCCAGCTCC-TGGCCCGCCGCT-3' (sense), 5'-TTCAAATGAGATTGTGGG-AAAATTGTC-3' (antisense); and GAPDH 5'-CCACCCATGG-CAAATTCATGGC-3' (sense), and 5'-TCTAGACGGCAGGTC-AGGTCCACC-3' (antisense).

### Immunohistochemical Analysis

Segments of atherosclerotic plaque were collected during surgery in formal saline (0.9% NaCl, 10% formaldehyde) and fixed for 24 hours. The tissues were paraffin embedded (Shandon Citadel 200; Lipshaw USA), and 5- to 8- $\mu$ m sections were cut (Leitz 1512 microtome; Weltzar GmbH). The sections were incubated in primary antibody against COX-1 (Cayman Chemical), COX-2 (Cayman Chemical), anti-smooth muscle cell  $\alpha$ -actin (Sigma Chemical), or HAM56 (DAKO) for 1 hour at room temperature. The COX-1 monoclonal antibody cross-reacts with both human and ovine COX-1 but does not cross-react with COX-2 from any species. The COX-2 polyclonal antibody was generated against amino acids 567 to 599 in the C terminus of human COX-2, a sequence that is unique to COX-2. This antibody does not cross-react with COX-1 from any species. After washing in PBS, the slides were incubated in the secondary biotinylated antibody, and the immunocomplex was visualized with use of the diaminobenzidine chromogen (ABC Complex, Vectastain Elite kit; Vector Laboratories). The presence of COX-2 in VSMCs was confirmed with immunofluorescence confocal microscopy. The sections were incubated with the COX-2 primary mouse antibody and an  $\alpha$ -actin rabbit antibody. These sections were incubated with a Texas Red-labeled anti-rabbit IgG (Vector Laboratories) and with a fluorescein isothiocyanate (FITC)-

labeled anti-mouse IgG (Vector Laboratories). Imaging was performed with an Axioplan LSM510 confocal microscope (Karl Zeiss)

### COX-1 Activity in Whole Blood

Serum TXB<sub>2</sub> was assayed by allowing whole blood to clot in nonsilicized glass tubes at 37°C for 1 hour.<sup>32</sup> Serum was separated through centrifugation at 1000g for 10 minutes. TXB<sub>2</sub> levels were measured with enzyme immunoassay (R and D Systems Europe).

### COX-2 Activity in Whole Blood

Blood was drawn into tubes containing 200  $\mu$ mol/L aspirin and 10 IU/mL sodium heparin (final concentrations) 2 hours after drug administration. Aliquots (1 mL) of whole blood were incubated in the presence and absence of 10  $\mu$ g/ $\mu$ L lipopolysaccharide (LPS; bacterial endotoxin derived from *Escherichia coli* O26:B6; Sigma Chemical) for 24 hours at 37°C. Plasma was separated by centrifugation at 1000g for 10 minutes and assayed for PGE<sub>2</sub> by enzyme immunoassay (R and D Systems Europe). The induced PGE<sub>2</sub> is due to the expression of COX-2 in monocytes in the whole blood.<sup>32</sup>

### Urinary Eicosanoid Excretion

Urinary metabolites of PGI<sub>2</sub> (2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub> ) and TXA<sub>2</sub> (11-dehydro-TXB<sub>2</sub>) and urinary 8-iso-PGF<sub>2 $\alpha$</sub>  were measured with gas chromatography/mass spectrometry as previously described.<sup>33,34</sup>

### Statistical Analysis

The numbers of patients were selected on the basis of the detection of a  $\geq 50\%$  reduction in 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  formation, as seen with nimesulide in normal subjects.<sup>21</sup> The data are expressed as mean  $\pm$  SEM. For the comparison of normal subjects with atherosclerotic patients, the data were analyzed with an unpaired Student's *t* test. For samples for the same subjects over time, the data were analyzed with Friedman's nonparametric 2-way ANOVA with subsequent paired analysis where appropriate. For comparisons between treatments, the data were analyzed with Kruskal-Wallis 1-way ANOVA with subsequent nonpaired analysis between groups.

## Results

### COX Isoform Expression in Atherosclerotic Plaque

COX-2 mRNA expression was not detected in 5 normal arterial sections that were obtained postmortem from young individuals who had no gross or microscopic evidence of atherosclerosis. However, all 10 samples analyzed from patients with atherosclerosis showed COX-2 mRNA expression. COX-1 mRNA was expressed in both normal and atherosclerotic sections but appeared to be induced in atherosclerotic tissue (data not shown).

Sections of atherosclerotic plaque were analyzed for COX-1 and -2 protein through immunohistochemistry ( $n=10$ ). The areas of atherosclerotic plaque were distinguishable with light microscopy by the presence of fatty streaks, foam cells, calcification, and cellular proliferation. This was confirmed with staining for proliferating VSMCs with anti- $\alpha$ -actin<sup>35</sup> and for macrophages with HAM56.<sup>36</sup> In the normal vessel, there was constitutive and diffuse expression of COX-1 protein in the adventitia and media but no detectable COX-2. In contrast, both COX-1 and -2 were expressed in atherosclerotic plaque.

### Immunofluorescent Microscopic COX-2 Expression in Infiltrating Macrophages and VSMCs

The labeling of COX-2 with Texas Red demonstrated COX-2 expression that corresponded to regions that also stained

green for smooth muscle cells (with FITC-labeled anti- $\alpha$ -actin). It is worth noting that not all of the VSMCs stained for COX-2 (Figure 1).

### Inhibition of COX-1 and COX-2 Ex Vivo

Blood was obtained for prostaglandin determinations at 1 to 2 hours after dosing to confirm the selectivity of nimesulide for COX-2. Serum TXB<sub>2</sub>, an assay of COX-1 activity, was markedly suppressed with aspirin ( $239.8 \pm 22.5$  to  $24.2 \pm 2.2$  ng/mL,  $P < 0.001$ ) and with aspirin plus nimesulide (to  $18.0 \pm 1.04$  ng/mL,  $P < 0.001$ ). In contrast, serum TXB<sub>2</sub> was little affected by nimesulide when administered alone ( $217.08 \pm 18.79$  ng/mL). The induction of PGE<sub>2</sub> after the incubation of whole blood with LPS ex vivo was used as an assay of COX-2 activity. Aspirin had no effect on this assay (as expected, because it is rapidly hydrolyzed) (from  $25.4 \pm 3.28$  to  $27.35 \pm 4.19$  ng/mL). In contrast, nimesulide markedly suppressed LPS-induced PGE<sub>2</sub> formation (from  $25.4 \pm 3.28$  to  $5.01 \pm 1.04$  ng/mL).

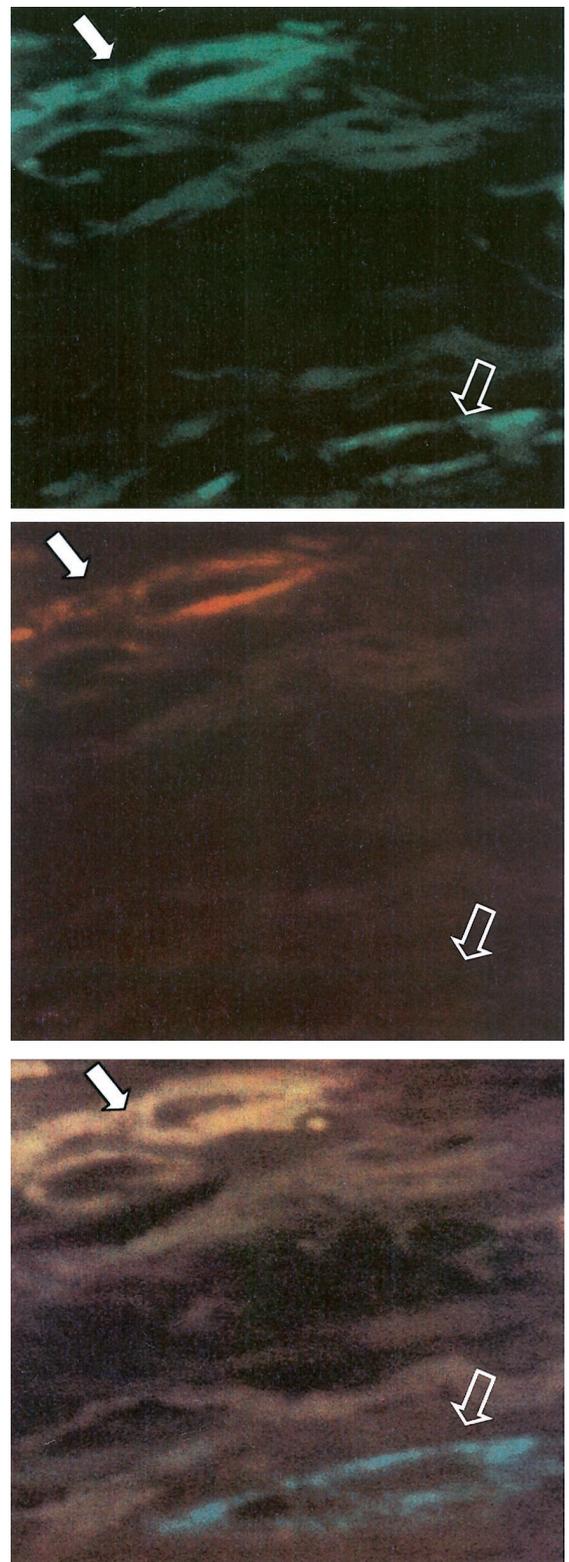
### Urinary Eicosanoid Excretion in Atherosclerosis: The Effect of Aspirin and Nimesulide

Urinary metabolite levels were measured in 18 patients with cardiovascular disease before they were given any drug. We also studied normal healthy volunteers (mean age  $35 \pm 5$  years, 6 men and 6 women), all of whom were nonsmokers and had no history of cardiovascular disease. Compared with normal healthy volunteers ( $n = 12$ ), urinary excretion of 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  was markedly elevated in these patients ( $594 \pm 156$  versus  $130 \pm 22$  pg/mg creatinine,  $P < 0.05$ ). There also were significant increases in urinary 11-dehydro-TXB<sub>2</sub> ( $3211 \pm 533$  versus  $679 \pm 63$  pg/mg creatinine,  $P < 0.001$ ) and 8-iso-PGF<sub>2 $\alpha$</sub>  ( $536 \pm 63$  versus  $250 \pm 21$  pg/mg creatinine,  $P < 0.01$ ).

The excretion of urinary metabolites before and after nimesulide but before surgery is shown in the Table. Nimesulide reduced urinary 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  by  $46 \pm 5\%$  ( $n = 8$ ,  $P < 0.01$ ) but had no significant effect on 11-dehydro-TXB<sub>2</sub> excretion or 8-iso-PGF<sub>2 $\alpha$</sub> . Also shown in the Table are the data for patients who were taking aspirin and for patients who were given the combination of aspirin and nimesulide. As expected, urinary 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  and 11-dehydro-TXB<sub>2</sub> were reduced in patients who were taking aspirin. The addition of nimesulide had little further effect and in particular did not reduce 11-dehydro-TXB<sub>2</sub> compared with aspirin alone. Although there was a modest reduction in 8-iso-PGF<sub>2 $\alpha$</sub>  excretion with nimesulide, this was not statistically significant.

### Effect of Nimesulide and Aspirin After Surgery

After surgery, there was a marked increase in the urinary excretion of 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  but not in the urinary excretion of 8-iso-PGF<sub>2 $\alpha$</sub>  (Figure 2). Nimesulide had no effect on the rise in urinary 11-dehydro-TXB<sub>2</sub> levels after surgery (Figure 2A), whereas aspirin blunted this increase. It is worth noting, however, that there still was an increase in urinary 11-dehydro-TXB<sub>2</sub> despite prior treatment with aspirin and that this was unaffected by the addition of nimesulide.



**Figure 1.** Expression of COX-2 in VSMCs. Immunofluorescent staining of atherosclerotic plaque for smooth muscle cells with anti-mouse smooth muscle cell  $\alpha$ -actin antibody counterstained with FITC-labeled mouse IgG (green) (top) and for COX-2 with anti-rabbit COX-2 antibody counterstained with a Texas Red-labeled rabbit IgG (middle). Bottom, Dual staining of COX-2 and smooth muscle cells (orange). Filled arrows identify a smooth muscle cell that stains for COX-2. Open arrows show a smooth muscle cell in which no COX-2 was detected.

### Urinary Excretion of Prostaglandin Metabolites and 8-isoPGF<sub>2α</sub> in Patients With Atherosclerosis Before Surgery

	Before Nimesulide (n=9)	Nimesulide (n=9)	Aspirin (n=14)	Aspirin+Nimesulide (n=6)
11-Dehydro-TXB <sub>2</sub>	2678±694	2110±282	1004±266†	1195±591*
2,3-dinor-6-keto-PGF <sub>1α</sub>	378±182	182±45†	241±53	100±34†
8-Iso-PGF <sub>2α</sub>	429±77	324±34	484±57	349±114

Patients taking aspirin either continued to take aspirin (n=8) or nimesulide was added to their treatment.

\* $P < 0.05$ , † $P < 0.01$  vs patients on no treatment (before nimesulide).

There also was a marked increase in 2,3-dinor-6-keto-PGF<sub>1α</sub> after surgery, which persisted for longer than the rise in 11-dehydro-TXB<sub>2</sub> (Figure 2B). Nimesulide markedly suppressed urinary 2,3-dinor-6-keto-PGF<sub>1α</sub> levels, particularly on the second day after surgery (1016±491 versus 3010±1120 pg/mg creatinine,  $P < 0.05$ , n=8). A further reduction in urinary 2,3-dinor-6-keto-PGF<sub>1α</sub> levels was seen with the addition of aspirin (465±119 pg/mg creatinine). None of the treatments significantly altered the excretion of 8-iso-PGF<sub>2α</sub> after surgery (Figure 2C).

### Discussion

The results of studies in normal subjects suggest that platelet COX-1 is the major source of TXA<sub>2</sub> in humans. Thus, platelet-specific preparations of aspirin with no effect on vascular COX maximally suppress TXA<sub>2</sub> formation,<sup>8</sup> whereas the selective inhibition of COX-2 has very little effect.<sup>21–23</sup> The results of similar studies with regular- and low-dose aspirin suggest that platelet COX is also a major source of the increased TXA<sub>2</sub> biosynthesis seen in patients with atherosclerosis, possibly reflecting enhanced platelet activity.<sup>5</sup> However, although low-dose aspirin is relatively selective for platelet COX, all regular doses of aspirin inhibit COX in tissues.<sup>37</sup> Indeed, TXA<sub>2</sub> may be generated by several cell types in vascular tissue, including monocytes, where either COX-1 or -2 may be responsible.<sup>9</sup> Thus, it has been suggested that tissue COX-2 may be the source of the persistent TXA<sub>2</sub> generation seen in patients with unstable angina who were taking aspirin,<sup>38</sup> in particular because this COX isoform is less sensitive to aspirin.<sup>39</sup>

Although COX-1 may be the primary source of TXA<sub>2</sub> in normal subjects, data from several studies show that COX-2 is the major source of endogenous PGI<sub>2</sub>. Thus, COX-2 inhibition markedly reduces the excretion of PGI<sub>2</sub> metabolites in normal volunteers.<sup>21–23</sup> However, it is not known whether COX-2 is responsible for the increased PGI<sub>2</sub> formation seen in atherosclerosis. Recent data suggest that COX-1 may also be induced and may be responsible for prostaglandin formation at sites of inflammation.<sup>29,30</sup>

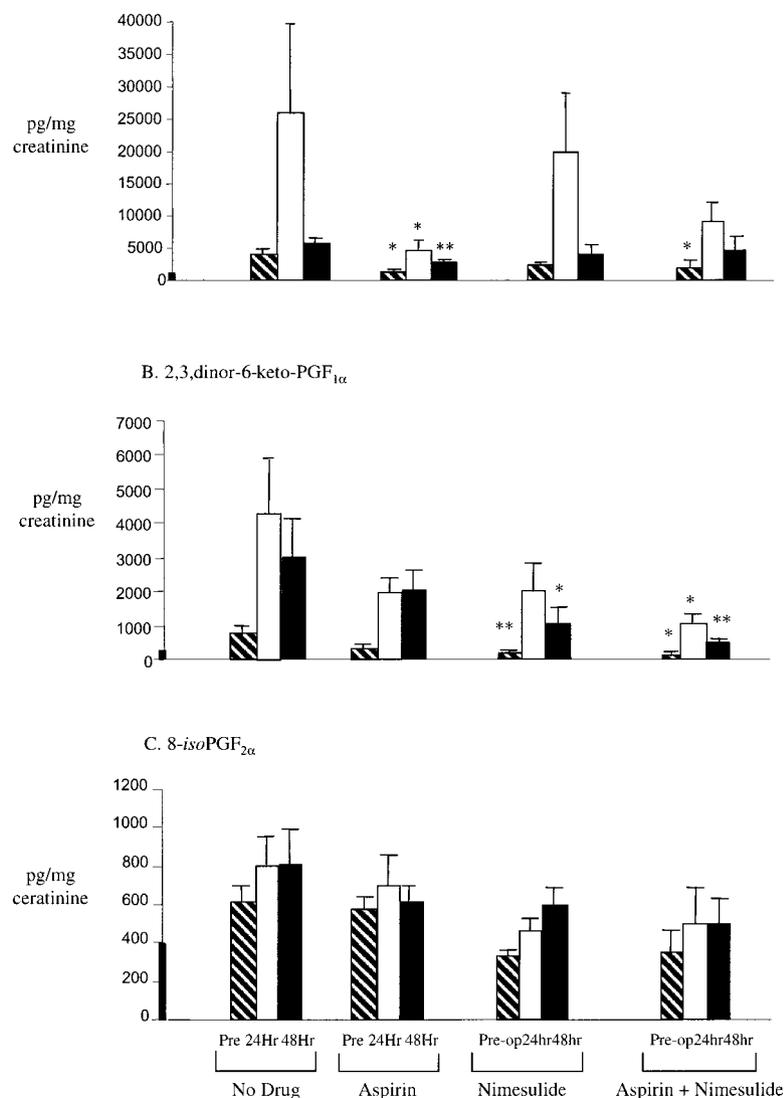
We showed through several approaches that COX-2 was induced in atherosclerotic plaque and that this was in part responsible for the increase in PGI<sub>2</sub> biosynthesis seen in patients with atherosclerosis. Thus, COX-2 mRNA was found in the atherosclerotic but not the normal blood vessels. Immunohistochemical studies localized the COX-2 expression to VSMCs and inflammatory cells, as reported previously.<sup>40</sup> Perhaps as a result

of the surgery, there was no endothelium evident in the sections, so it was not possible to evaluate COX isoform expression in endothelial cells. In addition to COX-2, product formation, mRNA expression, and immunohistochemical studies (not shown) provided evidence that COX-1 was also induced in atherosclerotic tissue.

The relative contribution of COX isoforms to prostaglandin generation was studied through an examination of the effects of nimesulide on eicosanoid formation. We have shown previously that nimesulide is selective for COX-2 at the dose used in this study.<sup>21,41</sup> Nimesulide had no effect on gastric COX activity or systemic TXA<sub>2</sub> formation while it suppressed LPS-induced PGE<sub>2</sub> formation.<sup>41</sup> Selectivity was confirmed in the present study in that nimesulide had little effect on serum TXB<sub>2</sub>, an assay of COX-1 activity, whereas it markedly suppressed LPS-induced PGE<sub>2</sub>, an assay of COX-2 activity.<sup>32</sup>

Nimesulide reduced the urinary excretion of 2,3-dinor-6-keto-PGF<sub>1α</sub> in patients with atherosclerosis by nearly 50% before surgery and to a similar extent after surgery. This finding suggests that the increased PGI<sub>2</sub> formation in part reflects COX-2 expression. However, urinary 2,3-dinor-6-keto-PGF<sub>1α</sub> was not reduced to the low levels seen in normal subjects taking aspirin<sup>8,37</sup> either before or after surgery. Thus, the increase in PGI<sub>2</sub> biosynthesis seen in atherosclerosis appears to reflect the activity of both COX isoforms. In contrast, nimesulide had little effect on urinary 11-dehydro-TXB<sub>2</sub>, the principal enzymatic metabolite of TXA<sub>2</sub>, either before or after surgery. Aspirin had a very profound effect, but despite >95% inhibition of platelet COX, there still was an increase in urinary 11-dehydro-TXB<sub>2</sub> in the patients taking aspirin. These findings suggest that both platelet and tissue COX-1 contribute to the increase in TXA<sub>2</sub> biosynthesis in patients with atherosclerosis.

An important question that concerns COX-2 inhibitors is whether the selective reduction of PGI<sub>2</sub> increases the risk of atherosclerosis. The role of PGI<sub>2</sub> in vivo is not clear. Although PGI<sub>2</sub> is a potent inhibitor of platelets,<sup>7</sup> the endogenous plasma levels are well below the threshold for a systemic antiplatelet effect.<sup>11</sup> However, disruption of the PGI<sub>2</sub> receptor in mice increases the risk of thrombosis.<sup>42</sup> Moreover, a recent study that showed greater efficacy in stroke prevention with a lower dose of aspirin suggests a role for endogenous PGI<sub>2</sub>.<sup>43</sup> Thus, the reduction in PGI<sub>2</sub> formation seen with a COX-2 inhibitor in the presence of normal TXA<sub>2</sub> formation may place patients at an increased risk of thrombosis. However, it should be emphasized that the findings for the present study group of severely diseased patients may not be applicable to patients with in more modest



**Figure 2.** Prostaglandin metabolite excretion after revascularization surgery in patients with atherosclerosis before (Pre-op, hatched columns) and at 24 hours (open columns) and 48 hours (filled columns) after surgery. Data are mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$  compared with patients taking no drug. Normal values for variable are shown in shaded areas on y axes.

disease state. Moreover, it is worth noting that in the present study, there was no further increase in TXA<sub>2</sub> formation while the patients were taking nimesulide even after the stimulus of surgery. TXA<sub>2</sub> is in large part derived from platelets, and increased TXA<sub>2</sub> formation is a marker of platelet activity.<sup>44,45</sup> Therefore, we saw no evidence that the reduction in PGI<sub>2</sub> enhanced platelet activity *in vivo*.

Indeed, given its expression in proliferating VSMCs, it is possible that the COX-2 activity contributes to the progression of atherosclerosis. COX-2 limits cell death in several tissues, including cardiomyocytes<sup>24</sup> and epithelial cancers,<sup>46</sup> and so may promote VSMC growth. COX-2 expression has also been shown to induce metalloproteinases,<sup>46</sup> which are enzymes involved in cell migration and destabilization of the atherosclerotic plaque.<sup>3,4</sup> Thus, COX-2 expression may contribute to the VSMC proliferation and migration that are hallmarks of early atherosclerosis. Moreover, there is evidence that COX-2 expression occurs early in the development of atherosclerosis in apoE-deficient mice.<sup>47</sup>

We also found a marked increase in isoprostane generation in patients with atherosclerosis, which is consistent with previous studies.<sup>13</sup> Both COX isoforms, and in particular COX-2, have been shown to generate 8-iso-PGF<sub>2α</sub> *in vitro*.<sup>14,15</sup> However, none

of the treatments significantly modified isoprostane formation, demonstrating that isoprostanes are not generated enzymatically in atherosclerosis. These data are consistent with studies of antioxidants that demonstrate isoprostane formation in atherosclerosis reflects oxidant injury.<sup>48</sup>

Our results are in agreement with recent evidence of COX-2 expression in atherosclerosis.<sup>40,49</sup> However, our results suggest that both isoforms are expressed and, moreover, that both contribute to the increase in PGI<sub>2</sub> biosynthesis seen in patients with atherosclerosis. In contrast, the increase in TXA<sub>2</sub> formation reflects COX-1 activity, probably as a consequence of enhanced platelet activation.

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