Angioplasty Increases Coronary Sinus F2-Isoprostane Formation: Evidence for In Vivo Oxidative Stress During PTCA

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OBJECTIVES Isoprostanes, stable end-products of oxygen free radical mediated–lipid peroxidation, were measured in the coronary vessels during percutaneous transluminal coronary angioplasty (PTCA) to provide direct evidence for enhanced oxidative stress in a local milieu in vivo.

BACKGROUND Percutaneous transluminal coronary angioplasty is associated with complications such as myocardial stunning and accelerated restenosis, which at least in part are mediated by oxygen free radicals. Because isoprostanes are markers of oxidant stress and potent vasactive compounds, the formation of which is not inhibited by aspirin treatment in vivo, it is possible that these mediators are increased locally during PTCA.

METHODS In 12 coronary artery disease patients who were given aspirin and ticlopidine, blood samples from coronary sinus were taken immediately before and immediately upon balloon deflation during PTCA. Isoprostane F2α-III, isoprostane F2α-VI, and TxB2 were quantified after extraction and chromatography using a stable dilution isotope gas chromatography/mass spectrometry assay.

RESULTS Coronary sinus and left main coronary artery levels of iPF2α-III and iPF2α-VI at baseline were (mean ± SEM) 40 ± 9 pg/ml and 115 ± 10 pg/ml, respectively. The TxB2 levels were undetectable. Following PTCA, isoprostane levels markedly increased (mean ± SEM): iPF2α-III, 125 ± 12 pg/ml (p < 0.001); iPF2α-VI, 295 ± 20 pg/ml (p < 0.001), whereas TxB2 levels remained undetectable.

CONCLUSIONS These results indicate that PTCA induces coronary sinus increase in F2α-isoprostane formation, and they also provide direct evidence for enhanced oxidative stress in a local milieu in vivo. Thus, an increased F2α-isoprostane formation could play a role in the pathogenesis of some PTCA-associated untoward events. (J Am Coll Cardiol 2001;37:76–80) © 2001 by the American College of Cardiology
Methods

Patients. Twelve consecutive subjects (mean age 57 years; range 45 to 70 years) were enrolled with the following characteristics: 1) stable angina pectoris with an exercise treadmill test positive for ischemia within the preceding six months; 2) single-vessel coronary artery defined as >70% stenosis in left anterior descending coronary artery deemed approachable by PTCA on previous diagnostic coronary angiography; 3) lack of angiographic features suggestive of an "active" lesion (defined as a lesion with an intracoronary filling defect suggestive of stenosis-related thrombus); and 4) no conduction defects on the electrocardiogram (ECG). All patients were taking aspirin (160 mg/day) for at least four weeks before the procedure, and ticlopidine (500 mg/day) for at least 48 h before the procedure. Other medications for the treatment of angina pectoris (calcium channel blockers, beta-blockers and nitrates) were continued. As controls, four patients (all men, mean age 58 years; range 46 to 70 years) undergoing cardiac catheterization for angiography; 3) lack of angiographic features suggestive of an "active" lesion (defined as a lesion with an intracoronary filling defect suggestive of stenosis-related thrombus); and 4) no conduction defects on the electrocardiogram (ECG). All patients were taking aspirin (160 mg/day) for at least four weeks before the procedure, and ticlopidine (500 mg/day) for at least 48 h before the procedure. Other medications for the treatment of angina pectoris (calcium channel blockers, beta-blockers and nitrates) were continued. As controls, four patients (all men, mean age 58 years; range 46 to 70 years) undergoing cardiac catheterization for angiography were studied. The study was approved by the ethical committee of the hospital board, and all patients signed an informed consent.

PTCA and sample acquisition. All 12 patients underwent PTCA via a femoral approach. Briefly, they were premedicated with diazepam and diphenhydramine. Heparin (5,000 IU) was given as a bolus at the beginning of the procedure and additional boluses were injected if necessary to maintain an activated coagulation time ≥250 s. Diagnostic coronary angiography was performed using a non-ionic contrast media (Omnipaque, Nycomed Imaging AS). Five contrast injections in different X-ray projections were used for the left coronary artery and two contrast injections for the right coronary artery (total 42 ml of nonionic contrast media). At the end of the diagnostic procedure, a 6F NIH catheter was positioned in the distal coronary sinus via the right internal jugular vein, and a 7F Judkins guide catheter was positioned in the ostium of the left main coronary artery to start the PTCA. For each patient, basal contemporary samples were obtained at this time from the coronary sinus and from the left main coronary artery. After flushing with saline and discarding 5 ml of blood, from each catheter a 10-ml sample was withdrawn into a second syringe containing heparin (500 IU/ml) and EDTA (2 mmol/liter). The EDTA was used to prevent the artifactual generation of eicosanoids during the sampling procedure. Next, the intracoronary wire was positioned distally in the left anterior descending artery, and the balloon catheter was advanced across the stenosis and inflated for 120 s at the nominal balloon inflating pressure. Immediately upon balloon deflation, after flushing with saline and discarding 5 ml of blood, a 10-ml blood sample was taken from the coronary sinus catheter as was done with the first sample. There were no acute vessel closures and no patients experienced complicating myocardial infarction, or significant bleeding. There were no stent implantations. Coronary angiography was then performed to ensure a satisfactory angioplasty result.

Patients undergoing diagnostic coronary angiography before valvular replacement operation were studied as controls. Blood samples were taken from the coronary sinus and the left main coronary artery after the performance of coronary angiography. No complications related to the procedure occurred.

Analyses were performed without knowledge of the location within the coronary artery from which the blood originated.

Biochemical analysis. Blood samples were centrifuged at 3,000 rpm for 10 min. Plasma was collected and added with 10 μg [3H]-arachidonic acid in order to detect any artifactual formation of the isoprostanes of interest, then stored at −80°C. Samples were shipped in dry ice and received within 24 h. Artifactual generation of IPs was monitored by measuring formation of [3H]-iPF2α-III or [3H]-iPF2α-VI. All of the gas chromatography/mass spectrometry (GC/MS) analyses were performed on a Fisons MD-800 mass spectrometer interfaced with a Fisons 800 GC and an AS-800 autosampler as previously described (15–18,24). The mass spectrometer was operated in the negative ion chemical ionization mode using ammonia as the moderating gas (15–18,24). Standard validation criteria as well as interassay and intra-assay variability for all the following methodology described have been reported elsewhere (24–26). The iPF2α-III was analyzed as pentafluorobenzyl ester (PFB)/ter-butylidemethylsilyl ether derivative (15–18,24,25), while iPF2α-VI was analyzed as PFB/trimethylsilyl ether derivative (24,25). TxA2, measured as its stable hydrolysis product TxB2, was analyzed as the PFB/ter-butyldimethylsilyl ether derivative (15–18,24).

Statistical analysis. All data are expressed as mean ± SEM. Data were compared by use of appropriate t-test for paired data, and by a one-way analysis of variance. Simple regression analysis was performed to analyze the relation between iPF2α-III and iPF2α-VI. Differences were considered significant at a value of p < 0.05.
RESULTS

Twelve patients underwent elective PTCA. The clinical characteristics of the population study are described in Table 1. Ten patients had a history of cigarette smoking, two had high blood pressure, eight had total plasma cholesterol levels greater than 200 mg/dl, and four had type II diabetes mellitus. No complications were encountered during PTCA. Angiography of the target coronary artery immediately after postdeflation sample acquisition showed no significant residual stenosis with normal contrast runoff.

Table 2 reports on the levels of TxB2, iP2α-III and iP2α-VI at baseline in patients undergoing PTCA. Samples taken from the coronary sinus before the balloon inflation had TxB2 values below the detection limit of the assay (Table 2). Levels of iP2α-III and iP2α-VI were 40 ± 9 and 115 ± 10 pg/ml, respectively (Table 2). By measuring [2H₈]-iP2α-III or [2H₈]-iP2α-VI levels in each sample (data not shown), no artifactual generation of iPs was detected. To assess whether the site of the sampling in the coronary artery was important for the baseline values observed, additional samples were taken from the left coronary artery. No significant difference in iPs and TxB2 levels was observed between samples taken from the coronary sinus or the left coronary artery (Table 2). In contrast, all of the post-PTCA samples taken from the coronary sinus showed increased amounts (mean ± SEM) of both iP2α-III (125 ± 12 pg/ml, p < 0.001) and iP2α-VI (295 ± 20 pg/ml, p < 0.001) (Fig. 1). No increase in TxB2 was detected in any of the subjects after PTCA, indicating that aspirin had completely suppressed platelet cyclooxygenase enzyme activity in these patients. A significant correlation existed between the levels of the two iPs (r = 0.68, p < 0.05).

Table 1. Clinical Characteristics of the Patients Undergoing PTCA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (n)</th>
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<tbody>
<tr>
<td>Total population</td>
<td>12</td>
</tr>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>Men (n)</td>
<td>11</td>
</tr>
<tr>
<td>Cigarette smoking (n)</td>
<td>10</td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>4</td>
</tr>
<tr>
<td>High blood pressure (&gt;200 mg/dl) (n)</td>
<td>2</td>
</tr>
<tr>
<td>High cholesterol (n)</td>
<td>8</td>
</tr>
<tr>
<td>Previous MI (n)</td>
<td>6</td>
</tr>
<tr>
<td>Positive ETT (n)</td>
<td>12</td>
</tr>
</tbody>
</table>

ETT = exercise treadmill test within six months before PTCA; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.

Table 2. Levels of TxB2, iP2α-III and iP2α-VI in the Coronary Sinus and in the Left Main Coronary Artery Before PTCA

<table>
<thead>
<tr>
<th></th>
<th>Coronary Sinus (n = 12)</th>
<th>Left Main Coronary Artery (n = 12)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>TxB2 (ng/ml)</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>iP2α-III (pg/ml)</td>
<td>40 ± 9</td>
<td>45 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>iP2α-VI (pg/ml)</td>
<td>115 ± 10</td>
<td>105 ± 12</td>
<td>NS</td>
</tr>
</tbody>
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ND = not detectable; NS = not significant.

DISCUSSION

Isoprostanes and PTCA. The iPs are prostaglandin isomers formed by a free radical catalyzed attack of phospholipid-containing arachidonic acid in situ in mem-

Figure 1. Coronary sinus levels of iP2α-III (A) and iP2α-VI (B) before and after elective PTCA (n = 12). *p < 0.001.

To assess the potential contribution of the catheter per se during angiography, we monitored the coronary levels of iPs in four patients undergoing cardiac catheterization for valvular heart disease. At the end of the diagnostic procedure, two samples from these patients were collected, one in the coronary sinus and one in the left main coronary artery, as was done for the cohort of PTCA patients. Levels of the two iPs were similar to the levels observed in the patients undergoing PTCA at baseline before balloon inflation (Table 3).
suggest that mediators not inhibited by aspirin may also play a role in some of the complications of PTCA. The finding that PTCA triggers an increase in coronary sinus isoprostane levels, whose actions are not inhibited by aspirin, provides further insight into the pathophysiology of PTCA-induced vasospasm and may have important implications in patients undergoing interventions that produce coronary vascular injury. Interestingly, intracoronary administration of iPF₂α-III has been shown to cause in vitro vasoconstriction in porcine and bovine coronary arteries with an EC₅₀ of 0.5 and 1 μmol/liter, respectively (21). These concentrations are similar to the intracoronary levels of iPF₂α-III that we found after PTCA. Thus, the constriction often observed in the distal segment of the instrumented vessel might be due to the formation and translocation “downstream” of this vasoactive prostaglandin isomer formed as result of oxidative stress.

Furthermore, PTCA-induced plaque rupture with the exposure of cellular constituents it is known to increase in vivo platelet activation and adhesion, which may play a role in some of the sequelae of this procedure. It has been reported that iPF₂α-III increases in vitro platelet activation and adhesion with a EC₅₀ similar to the one reported above, and that both effects are prevented by thromboxane receptor antagonist but not by aspirin (22,23). Thus, we suggest that this isoeicosanoid should be added to the complex list of biologically active compounds formed after PTCA.

**Conclusions.** The present investigation demonstrates for the first time an increase in iPs generation, markers of in vivo lipid peroxidation and oxidative stress, in the coronary sinus of patients after elective PTCA. Considering that their formation and biological activities are aspirin-insensitive and that the coronary sinus levels reached after PTCA are close to the ones that provoke coronary artery constriction in animals and increase human platelet activation and adhesion, we conclude that iPs could play a role in the untoward events of this procedure such as vasospasm. Further studies to assess the mechanism of PTCA-induced release of iPs as well as the impact of pharmacological interventions are warranted.

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**REFERENCES**

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