Angioplasty Triggers Intracoronary Leukotrienes and Lipoxin A₄
Impact of Aspirin Therapy

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Background. Percutaneous transluminal coronary angioplasty (PTCA) is a widely used and important method of reperfusioning coronary arteries. However, it is also associated with serious complications such as acute reocclusion and accelerated restenosis. The factors as well as the mechanisms involved in PTCA-associated complications remain to be fully elucidated. Because peptidoleukotrienes and lipoxins are potent vasoactive compounds, the formation of which is not inhibited by aspirin (ASA) treatment in vitro, it is possible that these eicosanoids are involved in PTCA-associated untoward events. To test this, we determined the intracoronary levels of peptidoleukotrienes and lipoxin A₄ (LXA₄) as well as thromboxane (TX) and 5S,12S-dihydroxyeicosatetraenoic acid (5S,12S-DIHETE; a product of double dioxygenation) after plaque rupture and evaluated the impact of ASA therapy.

Methods and Results. PTCA was performed on 12 patients with coronary artery disease, six undergoing ASA therapy and six without ASA therapy, for at least 2 weeks before PTCA. By means of a technique that permitted sampling of intracoronary blood at the plaque site in situ, samples were taken immediately before and 10 seconds after initiation of plaque rupture. Lipoxygenase (LO)-derived products, including LXA₄ and 5S,12S-DIHETE, and a marker of cyclooxygenase activity, i.e., TXB₂, were quantitated after extraction and chromatography using deuterium-labeled internal standards and electron capture negative ion chemical ionization mass spectrometry. Peptidoleukotrienes (LTC₄ and LTD₄) were quantitated after reverse-phase high-performance liquid chromatography coupled with radioimmunoassay. Intracoronary blood taken before PTCA showed no detectable levels of these eicosanoids (the minimum limits of detection were within the picomole range). In contrast, each of these LO products was detected after PTCA. Patients undergoing ASA treatment showed elevated levels of each LO product examined compared with those not receiving ASA. Eicosanoid levels were (mean±SEM): LTC₄, 7.10±1.22 ng/ml (ASA) versus 0.48±0.10 ng/ml; LTD₄, 4.92±0.56 ng/ml (ASA) versus 1.17±0.48 ng/ml; LXA₄, 24.98±4.11 ng/ml (ASA) versus 15.83±2.43 ng/ml; 5S,12S-DIHETE, 19.47±3.98 ng/ml (ASA) versus 11.98±1.83 ng/ml; TXB₂, complete blockage (ASA) versus 31.04±7.38 ng/ml (p<0.05 for LTC₄ and LTD₄). To distinguish between dilatation of whole blood versus dilatation of whole blood and atheroma for contribution of eicosanoids, we also monitored their formation in Gore-tex grafts. Upon balloon inflation, TXB₂ was generated, but LO products were not detected. In contrast, injection of platelet- and leukocyte-directed agonists within the graft led to both peptidoleukotriene and lipoxin formation.

Conclusions. The results indicate that PTCA triggers the intraluminal release of peptidoleukotrienes and LXA₄, and that ASA therapy enhances their appearance in intracoronary blood. In addition, they provide direct evidence for LO products (LTC₄, LTD₄, and LXA₄) in a local milieu in vivo. Moreover, the presence of the double dioxygenation product 5S,12S-DIHETE (a potential marker of 5- and 12-LO interactions) suggests that transcellular metabolic events can contribute to eicosanoid formation in vivo. (Circulation 1992;86:66–63)

Key Words • PTCA • eicosanoids • lipooxygenase • leukocytes • platelets • lipoxins

Percutaneous transluminal coronary angioplasty (PTCA) is an effective method of treating patients with symptomatic coronary artery disease.¹ PTCA achieves vessel dilatation, including both plaque rupture and lumen expansion, by stretching the vessel wall,² but exposure of the cellular constituents of the atherosclerotic plaque to circulating platelets and white cells during this procedure generates vasoactive and thrombogenic mediators, which may account for PTCA complications ranging from subacute restenosis

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Supported in part by National Institutes of Health grants AI-26714, GM-38765, and PO1-HL-36028 (C.N.S.). D.A.B. is a Research-Resident of the Brigham and Women's Hospital and Beth Israel Hospital. C.N.S. is an Established Investigator of the American Heart Association, a Pew Scholar in the Biomedical Sciences, and recipient of the Clifford M. Clarke Science Award from the Arthritis Foundation.

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Received September 18, 1991; revision accepted March 12, 1992.
to abrupt closure. Several studies have documented the role of cyclooxygenase-derived eicosanoids in defining the events before and after PTCA-induced coronary vascular injury. For example, thromboxane generation by activated platelets and during PTCA is inhibited by aspirin (ASA) and is responsible for vasoconstriction both at the site of arterial injury and distally at the level of the coronary arterioles, sites that control coronary vascular resistance, However, a significant amount of PTCA-induced vasospasm and platelet aggregation occurs in patients treated with ASA in doses that inhibit cyclooxygenase and thromboxane formation. Fischell et al showed that spontaneous vasoconstriction after PTCA in humans occurred routinely at and distal to the site of balloon dilatation despite pretreatment with ASA. Likewise, restenosis after PTCA has not been shown to be altered with ASA. These findings suggest that substances not inhibited by ASA may play an important role in vasospasm, thrombosis, and accelerated atherosclerosis associated with plaque rupture.

Lipoxygenase (LO)-derived eicosanoids (i.e., peptidoleukotrienes and lipoxins) have proved to be potent vasoactive products whose formation is not inhibited by ASA. Peptidoleukotrienes (LTs) (LTC₄ and LTD₄) generated via the 5-LO system are vasoconstrictors of both coronary and cerebral arteries, act to decrease cardiac output, and promote vascular permeability. Members of the lipoxin series display both unique structures and actions and can be generated either by individual cell types or via transcellular routes of metabolism (Reference 16, recently reviewed in Reference 17). Lipoxin A₄ (LXA₄) provokes changes in microcirculation with arteriolar dilatation, and prostanooid-independent dilatation of cerebral arterioles. It also stimulates the formation of prostacyclin by human endothelial cells and possesses counterregulatory properties. These counterregulatory actions include LXA₄ antagonism of LTD₄-induced vasoconstriction, inhibition of human neutrophil response in vitro, and blockage of LTB₄-induced inflammation in the hamster cheek pouch. Unlike either leukotrienes or lipoxins, which are generated from epoxide-containing intermediates, interaction between the 5-LO of human neutrophils and the 12-LO of platelets can also result in the biosynthesis of 5S,12S-dihydroxy-6,8,10,14-eicosatetraenoic acid (5S,12S-DihETE). This product of double dioxygenation is an isomer of LTB₄ and is a product of transcellular metabolism in vitro; however, its vasoactive properties and generation in vivo remained to be defined.

Because of the potential importance of these LO-derived eicosanoids and cell–cell interactions in vascular disease (reviewed in Reference 26) and in view of the PTCA-related sequelae that are not inhibited by ASA therapy, we monitored the intracoronary levels of peptidoleukotrienes, LXA₄, and 5S,12S-DihETE within the frame of PTCA and evaluated the impact of ASA therapy. The peptidoleukotrienes and LXA₄ were monitored because of their documented vasoactive properties, and 5S,12S-DihETE was evaluated as an indicator of transcellular eicosanoid metabolism.

Methods

Patients

Twelve subjects were identified with the following inclusion criteria: 1) stable angina pectoris with an exercise treadmill test positive for ischemia within the preceding 6 months; 2) single-vessel coronary artery defined as a >70% stenosis in either the right, left anterior descending, or left circumflex coronary arteries deemed approachable by PTCA on previous diagnostic coronary angiography; and 3) lack of angiographic features suggestive of an “active” lesion (defined as a lesion with an eccentric morphology or an intracoronary filling defect suggestive of stenosis-related thrombus). Six of the patients were receiving ASA therapy (325 mg every day for at least 2 weeks before enrollment), which was not interrupted the day of PTCA. The other six patients who were not taking ASA therapy before enrollment abstained from ASA treatment as well as nonsteroidal anti-inflammatory agents for at least 2 weeks before PTCA. Other medications for the treatment of angina pectoris (calcium channel blockers, β-blockers, and nitrates) were continued. Consent was approved by the Committee for the Protection of Human Subjects at the New England Deaconess Hospital and for sample analysis at the Brigham and Women’s Hospital through a discarded-materials protocol.

PTCA and Intracoronary Sample Acquisition

All 12 patients underwent PTCA via a femoral approach after informed consent was obtained. All patients were premedicated with diazepam and diphenhydramine. Heparin (10,000 units) was given as a bolus at the beginning of the procedure. After angiography of the target vessel with an 8F guiding catheter, a PTCA balloon catheter (Hydracross Taper, Baxter LIS Division, Irvine, Calif.) with balloon size chosen to approximate the normal lumen size of an uninvolved segment of the vessel was advanced over a 0.014-in. guide wire to a position immediately proximal to the stenosis. After the guide wire was removed from the lumen of the catheter, a plastic syringe was attached to the proximal hub of the catheter. A 5-ml sample was taken via the distal tip of the catheter and discarded. Next, a 10-ml sample was withdrawn into a second syringe containing heparin (500 units/ml), the LO inhibitor esculetin (400 µM), and indomethacin (400 µM), a cyclooxygenase inhibitor. These inhibitors were used to prevent the generation of eicosanoids during the sampling procedure. The guide wire was then reintroduced and advanced across the stenosis, and the balloon catheter was advanced over the guide wire across the lesion. Balloon inflation was performed for 60 seconds using inflation pressures that would ensure nominal balloon size. During the last 10 seconds of balloon inflation, the guide wire was removed. Immediately upon balloon deflation, a 5-ml sample was taken and discarded, and a second sample was drawn just distal to the area of stenosis ablation as was done with the first sample. Coronary angiography was then performed to ensure a satisfactory angioplastic result. All samples were immediately placed at 4°C in an ice bath, coded (ASA or no ASA), and stored at −70°C. Analyses were performed without knowledge of the location within the coronary artery from which the blood originated and whether or not the patient was on ASA.

Reagents

Reagents were essentially the same as in References 28 and 29. 19,19,20,20,20-pentadeuterolipoxin A₄...
methyl ester was the kind gift of Professor K.C. Nicolaou (Department of Chemistry, Research Institute of Scripps Clinic, La Jolla, Calif.). [H]5,5S,12S-DiHETE was prepared by biogenic synthesis from synthetic 5,6,8,9,11,12,14,15-[H]-octadecueroarachidonic acid purchased from Cayman Chemical (Ann Arbor, Mich.). Briefly, freshly isolated platelet suspensions were prepared and fractionated. The resulting supernatants containing 12-LO activity were combined, and indomethacin (100 μM final concentration) and [H]-arachidonic acid (5 mg) were added, incubated for 45 minutes at 37°C, and stopped with methanol (2 vol). [H]-12-HETE was recovered by ethyl acetate extraction and incubated (45 minutes at 37°C) with freeze-dried lysozyme obtained from human neutrophils displaying 5-LO activity. After ethyl acetate extraction, [H]-5S,12S-DiHETE was isolated by reverse-phase (RP) high-performance liquid chromatography (HPLC). The [H]-5S,12S-DiHETE gave physical properties including UV spectrum, HPLC retention, and prominent ions in its mass spectrum consistent with the reported characteristics of unlabeled 5S,12S-DiHETE.

**Analysis of Intraluminal Eicosanoids**

Samples were extracted from intraluminal materials by use of a method described for blood with 10 ng each of [H]-lipoxin analogue, [H]-thromboxane B2 (TXB2), and [H]-5S,12S-DiHETE as internal standards. Pentfluorobenzyl (PFB) ester/trimethylsilyl (TMS) ether derivatives were prepared and samples were taken to dryness with N2 and suspended in n-hexane for gas chromatography/mass spectrometry (GC/MS) analysis.

Electron capture negative ion chemical ionization (NICI)-GC/MS was performed with a Hewlett-Packard 5890 GC and a 5988A MS equipped and controlled with a 59970A HP workstation and data system. Injects (2 μl hexane) were made in the splitless mode and spectra were acquired in the NICI mode; selected ion monitoring (SIM) was used in the dwell times set at 300 msc for the six anions of interest (i.e., m/z 572 ([H]-LXA4 analogue); m/z 567 [LXA4]; m/z 585 [TXB2]; and m/z 589 ([H]-TXB2]; and m/z 479 [5S,12S-DiHETE]; and m/z 487 ([H]-5S,12S-DiHETE]), and the corresponding areas beneath each of these peaks were determined by integration using 59970A HP Chemstation software. In this GC system the PFB ester/TMS ether derivatives are well resolved with baseline separation of authentic standards. The PFB ester/TMS ether derivative of LXA4 eluted at 7.8–8.0 minutes before the α-hydroxy-LTB4 derivative. Likewise, the 5S,12S-DiHETE derivative eluted at 0.5–1.6 minutes before the LTB4 PFB ester/TMS ether derivative. For quantitation, standard curves were constructed using the eicosanoids and their respective deuterated internal standards. Linear regression analysis gave correlation coefficients of r=0.99 (LXA4) and r=0.95 (TXB2). The minimum limits of detection were 74.0±14.1 pmol for LXA4 and 114.9±25.9 pmol for TXB2. Recovery from the combined plasma plus packed cell fractions after NICI SIM was 93.4±4.8% for [H]-TXB2 and 88.6±3.4% for LXA4 (internal standards). These values were consistent with those recently determined with samples from peripheral blood.

Peptidoleukotrienes (LTC4, LTD4, and LTE4) were separated and quantitated after 1H solid-phase extraction using RP-HPLC. This HPLC system was calibrated using the retention times of synthetic LTC4, LTD4, and LTE4, and their quantities were determined by comparing peak areas obtained for calibrated standards. In addition, fractions (1 ml) from this HPLC were collected, dried under vacuum at 23°C, and suspended in phosphate buffer (200 μl of 10 mM solution, pH 7.4) containing 0.9% NaCl, 0.1% gelatin, 0.01 M EDTA, and 0.1% sodium azide for quantitation by radioimmunoassay (NEN, Boston).

**Ex Vivo Analysis Within a Gore-tex System**

Fresh peripheral blood was obtained from healthy volunteers (ages, 18–35 years) who denied taking ASA or other medications for at least 2 weeks before venipuncture. Venous blood (90 ml) was collected with heparin (500 units/ml). Aliquots (10 ml) were infused into nonendothelialized Gore-tex grafts (3 mm×30 cm, Gore+Assoc., Wakefield, Mass.) and equilibrated at 37°C. After each graft, a 7F balloon catheter (Mansfield Scientific, Inc., Flagstaff, Ariz.) was inserted and inflated to 7 atm for 10 seconds, and samples were removed through the balloon catheter into a syringe containing indomethacin and esculin (as above). Incubations were also carried out with injections of selected stimuli (10 min, 37°C). All samples were placed on ice, and eicosanoids were extracted and quantitated as above.

**Statistics**

Levels of significance, p, of the differences between ASA and untreated groups were calculated with a paired Student's t test. Values of p<0.05 were considered statistically significant.

**Results**

**Patients**

Twelve patients (mean age, 61 years; range, 40–78 years) underwent PTCA. There were no apparent differences with respect to either age, sex, prevalence of diabetes, previous myocardial infarction, distribution of coronary artery disease, or concomitant cardiac medications between the ASA- and non-ASA-treated groups (Table 1). No complications were encountered during PTCA. Angiography of the target coronary artery immediately after postdilation sample acquisition showed no significant residual stenosis with normal contrast run-off.

Representative SIM NICI-GC/MS chromatograms obtained for the quantitation of the PFB ester/TMS ether derivatives are given in Figure 1. The samples were analyzed for the ion current intensities of the respective base peaks at m/z 567 and 572 (for LXA4), m/z 585 and 589 (TXB2), and m/z 479 and 487 (5S,12S-DiHETE). The retention times were (mean±SEM): LXA4 (m/z 567), 13.28±0.06 minutes; TXB2 (m/z 585), 13.37±0.06 minutes; and 5S,12S-DiHETE (m/z 479), 12.52±0.08 minutes. Thus, the appearance of the anions at m/z 567, m/z 585, and m/z 479 with appropriate retention times is consistent with the release of LXA4, TXB2, and 5S,12S-DiHETE, respectively, in the intra-coronary lumen.
Table 1. Patient Characteristics

<table>
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<tr>
<th></th>
<th>Total population (n=12)</th>
<th>Aspirin-treated (n=6)</th>
<th>No aspirin (n=6)</th>
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<tr>
<td>Age (years) (mean±SD)</td>
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<td>58±14</td>
<td>64±11</td>
</tr>
<tr>
<td>Men (n)</td>
<td>8</td>
<td>4</td>
<td>4</td>
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<tr>
<td>History of tobacco use (n)</td>
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<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Diabetes (n)</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Previous MI (n)</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Positive ETT (n)</td>
<td>12</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>NYHA (mean±SD)</td>
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<td>1.0±0.0</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>KPS score (mean±SD)</td>
<td>8.6±0.6</td>
<td>8.8±0.4</td>
<td>8.4±0.5</td>
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<tr>
<td>Target lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>LCx</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OM1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>RCA</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

MI, myocardial infarction; ETT, exercise treadmill test within 6 months before PTCA; NYHA, New York Heart Association angina classification; KPS score, Karnofsky Performance Status score (see Reference 36); LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery; OM1, first obtuse marginal branch; RCA, right coronary artery.

Table 2 reports the levels of individual eicosanoids from ASA-treated and -untreated patients. Intracoronary samples taken before the balloon inflation proximal to the site of the plaque had no detectable amounts of LO-derived eicosanoid in any of the 12 samples. In three of the 12 patients, additional samples were taken distal to the atherosclerotic plaque immediately before balloon inflation. These included one from the ASA group and two from the untreated group. In each of these samples, no detectable quantities of LO products were measured (data not shown). Also, there were no detectable levels of TXB_2 in the intracoronary samples taken before balloon inflation. In contrast, the post-PTCA samples taken primarily distal to the site of the plaque obtained from all subjects gave detectable amounts of each of the eicosanoids examined; LTE_4, however, was not found in any samples (data not shown). Elevated levels of LO-derived products were obtained from samples from ASA-treated subjects compared with those obtained from the non-ASA-treated group (Table 2). TXB_2 was not detected in samples from the ASA-treated group, which can be taken as an index of cyclooxygenase inhibition.

To assess the potential contribution of whole blood in the production of eicosanoids after angioplasty, we monitored the generation of these products in nonendothelialized Gore-tex grafts filled with blood taken from healthy donors and subjected to either balloon inflation or leukocyte- and/or platelet-directed agonists (Table 3). Eicosanoids were not detected in samples in which anticoagulated whole blood was incubated within Gore-tex graft without stimuli or mechanical manipulation (data not shown). As expected, upon injection of thrombin, TXB_2 was generated. On the other hand, LXA_4, 5S,12S-DiHETE, and the peptidoleukotrienes were produced only when the samples were exposed to both thrombin and the chemotactic peptide formylmethionyl-leucine-phenylalanine.

Balloon inflation did not lead to the generation of detectable levels of 5S,12S-DiHETE in Gore-tex grafts (Table 3), suggesting that these two forms of activating components of whole blood (balloon inflation versus selected stimuli) are not comparable. Addition of the ionophore A23187 (5 µM) to whole blood ex vivo gives a TXB_2:LXA_4 ratio of ≈3:1, which is comparable to thrombin (1.0 IU), albeit the levels are ≈4 times lower. When isolated whole blood is incubated with A23187 (25 µM), evidence for only small amounts of 5S,12S-DiHETE has been presented compared with the levels of LTB_4 generated in the incubations as monitored by HPLC. The ionophore A23187 is a powerful stimulus for LTB_4 production in whole blood that bypasses receptor ligand activation. Thus, it appears that the profile and quantitative relation between eicosanoids generated is dependent not only on the stimulus or agonist but also on the cell types and materials present in the local milieu.

Discussion

In this paper, we report the intracoronary identification of LO-derived eicosanoids after plaque rupture induced by PTCA. Samples taken before balloon inflation that were immediately proximal to a stenosis did not reveal the presence of these eicosanoids. They were also not detected in samples from three individuals taken immediately distal to the site of the atherosclerotic plaque. In contrast, those taken distal to the plaque site after PTCA contained LO-derived eicosanoids, i.e., LXA_4, LTC_4, LTD_4, and 5S,12S-DiHETE (Table 2). In addition, patients undergoing ASA therapy had significantly higher levels of LTC_4 and LTD_4. These results are the first to document intracoronary levels of LO-derived eicosanoids and the impact of ASA therapy. Moreover, they provide direct evidence for the presence of LXA_4 and 5S,12S-DiHETE within a human vessel (Figure 1, Table 2), both of which are potential products of transcellular eicosanoid metabolism in human tissues.

LO products of arachidonic acid have been shown to play critical roles in a host of important physiological responses. In vitro evidence indicates that leukotrienes and lipoxins represent products of both intracellular and transcellular arachidonic acid metabolism with cell types known to be located within the coronary artery system (i.e., white cells, platelets, and endothelial cells) that can participate in the process of atherosclerosis. These forms of transcellular metabolism may represent important biological signaling events in both physiological and pathophysiological states because they can lead to the amplification of individual eicosanoids. Here, we obtained direct evidence for LTC_4, LTD_4, and LXA_4 within the intracoronary local milieu (Table 2). It should be noted that the lesions studied here did not appear to be traumatized upon positioning of the deflated catheter, because no obstruction of flow was observed. Thus, identification of these LO-derived eicosanoids in samples taken after stenosis, dilatation, and disruption and not in those taken before balloon inflation suggests that plaque rupture may be the stimulus triggering the appearance of these vasoac-
tive compounds. Taken together, these findings suggest that PTCA triggers the appearance of eicosanoids in the lumen of the coronary artery, which may be derived from 1) the atherosclerotic plaque itself, 2) the interaction of the released plaque debris with peripheral blood cells, and/or 3) activation of the peripheral blood by the

**TABLE 2. LXA₄, TXB₂, 5S,12S-DiHETE, and Peptidoleukotriene Levels Before and After PTCA in Patients: Impact of Aspirin Therapy**

<table>
<thead>
<tr>
<th></th>
<th>LXA₄</th>
<th>TXB₂</th>
<th>5S,12S-DiHETE</th>
<th>LTC₄</th>
<th>LTD₄</th>
</tr>
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<tr>
<td><strong>Aspirin</strong></td>
<td></td>
<td></td>
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<tr>
<td>Before PTCA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>After PTCA</td>
<td>24.98±4.11</td>
<td>ND</td>
<td>19.47±3.98</td>
<td>7.10±1.22*</td>
<td>4.92±0.56*</td>
</tr>
<tr>
<td><strong>No aspirin</strong></td>
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<tr>
<td>Before PTCA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>After PTCA</td>
<td>15.83±2.43</td>
<td>31.04±7.38</td>
<td>11.98±1.83</td>
<td>0.48±0.10</td>
<td>1.17±0.48</td>
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</tbody>
</table>

LXA₄, lipoxin A₄; TXB₂, thromboxane B₂; 5S,12S-DiHETE, 5S,12S-dihydroxy-6,8,10,14-eicosatetraenoic acid; PTCA, percutaneous transluminal coronary angioplasty; LT, leukotriene; ND, not detected. All results are expressed as ng/ml intracoronary sample.

Samples were collected immediately before and 10 seconds after balloon inflation into syringes containing heparin (500 units/ml), esculetin (400 μM), and indomethacin (400 μM) and placed on ice. Results represent mean±SEM of n=6 from each treatment group.

*p<0.05 compared with no aspirin group.
Balloon catheter. The absence of detectable quantities of LO-derived products before balloon inflation does not preclude the possible existence of either leukotrienes or lipoxins generated locally distal to the stenosis before angioplasty. Although the origin(s) of production and/or liberation of these LO products cannot be shown conclusively from the present results, recent evidence suggests that the cellular components of atherosclerotic plaques can generate LO-derived products. Monocytes, foam cells, and platelets are major cell types of atherosclerotic plaque, and messenger RNA for 15-LO colocalizes in the macrophage-rich regions in plaques from hyperlipidemic rabbits. When stimulated, human mononuclear cells generate LTC₄, LTB₄, and 5-HETE. Also, transformed foam cells have been demonstrated to produce LTC₄ and platelet–neutrophil interactions in vitro generate peptidoleukotrienes, lipoxins, and 5S,12S-DiHETE through individual transcellular pathways (see above; reviewed in References 17 and 26). Canine atherosclerotic plaques stimulated ex vivo produce both 12-HETE and 15-HETE, indicative of LO activities. LO-derived compounds are also generated in response to vascular trauma, and in this respect, balloon inflation has been demonstrated to produce vascular trauma. These studies taken together with the present results suggest that balloon inflation itself into the atherosclerotic plaque may trigger the liberation of vasoactive LO-derived eicosanoids within the coronary lumen.

In a second group of experiments to address this, balloon inflation into Gore-tex grafts filled with whole blood failed to generate detectable quantities of LO-derived eicosanoids (Table 3). This finding also suggests the participation of the atherosclerotic plaque. Thus, the appearance of LO-derived eicosanoids within the coronary artery may involve either 1) release of preformed eicosanoids or their precursors liberated from the plaque into blood during plaque rupture or 2) direct activation of peripheral blood components (i.e., platelets and neutrophils) by compounds or debris released from the plaque. Together, the results in Table 3 suggest that balloon inflation can stimulate TXB₂ production, presumably by mechanical activation of platelets, a finding consistent with a previous report. However, balloon inflation within Gore-tex grafts itself failed to generate peptidoleukotrienes, LXA₄, and 5S,12S-DiHETE in these samples, suggesting that the atheroma may participate in the production of LO-derived eicosanoids immediately after PTCA.

5S,12S-DiHETE is structurally similar to LTB₄ in that it is a 5,12-dihydroxy acid. Unlike LTB₄, however, it is not derived from the epoxide LTA₄ but is generated primarily via double dioxygenation of arachidonic acid by the sequential activities of the 5-LO and 12-LO (in either order). 5S,12S-DiHETE is believed to be a product derived solely from transcellular metabolic events. Although the bioactions of 5S,12S-DiHETE remain to be clarified, recent results indicated that it can desensitize the LTB₄ receptor of polymorphonuclear leukocytes. The identification of 5S,12S-DiHETE in intracoronary samples represents the first demonstration of this eicosanoid in vivo, which may then serve as a marker of transcellular eicosanoid metabolism. LTC₄ is the least vasoactive of the peptidoleukotrienes and has recently been reported to be rapidly eliminated as unmetabolized LTC₄ and as ω- and methyl terminus β-oxidation products. LTC₄ was not detected in the intracoronary blood samples. This may reflect either a lack of conversion from LTD₄ to LTE₄ within the sampling period or that rapid ω- and methyl terminus β-oxidation takes place, which renders the compounds undetectable with the present methods.

NICI-GC/MS analysis using quantitation by stable isotopic dilution has proved to be both a sensitive and selective method for quantitation of individual eicosanoids within complex biological matrices that has recently been developed for lipoxins. The [M-PFB]+ of the LXA₄ derivative is m/z 567, an anion that could also be obtained for [M-PFB]+ of the ω-20-hydroxy-LTB₄ derivative. When the PFB/TMS derivative of this LTB₄ metabolite was prepared, it eluted >7 minutes after the PFB ester/TMS ether derivative of LXA₄ (see “Methods”), indicating that, if present, ω-20-hydroxy-LTB₄ would not interfere with the detection of LXA₄. Along these lines, previous results with human whole blood indicate that LTB₄ added to whole blood is not ω-oxidized and converted to the 20-hydroxy metabolite. These results suggest that if LTB₄ is generated or liberated during plaque rupture, it may not be subject to ω-oxidation in the intracoronary milieu. LTB₄ was not quantitated in the present study; thus, its possible presence in the intracoronary milieu after plaque rupture awaits future studies.

The mass ratio of LXA₄ to peptidoleukotrienes appears to favor LXA₄. In addition, peptidoleukotrienes were elevated in a statistically significant fashion after ASA therapy (Table 2). These findings suggest that the increments in LO products observed with ASA therapy may reflect preferential metabolism of arachidonic acid by LO after inhibition of the cyclooxygenase with ASA therapy. This type of substrate “shunting” between cyclooxygenase to LO activities has been well documented with both isolated cell suspensions and tissues in vitro (see References 11 and 44).
LXA₄ suggests that the peptidoleukotriene/LXA₄ index may be relevant in intracoronary vascular events, particularly because LTC₄ and LTD₄ are potent vasoconstrictors, whereas LXA₄ has proved both to be a vasoconstrictor and to block LTD₄- and LTβ₁-induced responses in animal models.

The finding that PTCA triggers the intracoronary formation and release of vasoactive leukotrienes and lipoxins whose actions are not inhibited by ASA therapy may have important implications in patients undergoing interventions that produce coronary vascular injury. Acute vessel closure and subsequent restenosis after PTCA is thought to be mediated by thromboxane released by activated platelets at the site of plaque fracture that promotes coronary vasoconstriction and platelet aggregation. Despite ASA’s ability to block cyclooxygenase-derived eicosanoid coronary vasoconstriction in animal models of PTCA, it failed to prevent vasoconstriction both at and distal to the site of plaque fracture in 10 patients undergoing PTCA. Constriction in the distal segment of the instrumented vessel may be due to translocation “downstream” of other vasoactive arachidonic acid products such as leukotrienes formed at the site of PTCA. Lane and Bove have shown in an intact anesthetized canine preparation that a combination of balloon-induced endothelial injury and cyclooxygenase inhibition with indomethacin or meclofenamate resulted in coronary vasoconstriction, suggesting that other arachidonic acid-derived products such as leukotrienes C₄, D₄, and E₄ may play a role in PTCA-related complications or focal spasm of the coronary arteries.

Coronary flow reserve is transiently depressed, despite ASA pretreatment, in patients after successful PTCA, which is likely the result of vasoconstriction at the level of the intramyocardial arterioles. Intracoronary administration of LTC₄ and LTD₄ has been shown to cause coronary arteriolar constriction, and recently we have found that LTD₄ is a potent stimulus for the release of von Willebrand factor from human endothelial cells in culture. Thus, further studies to assess the mechanisms of PTCA-induced release of LO-derived eicosanoids as well as the impact of pharmacological interventions are warranted.

In the current study, we examine the intracoronary appearance of lipoxin A₄, thromboxane B₂, peptidoleukotrienes, and SS,12S-DiHETE before and after PTCA. A complete profile of eicosanoids and their temporal relation would be insightful as to the relative production of the individual products. Here, we limited the measurements to selected representatives shown to be vasoactive compounds from the lipoxigenase pathways. Further studies will widen the scope of other eicosanoids that may be generated by the atherosclerotic plaque. In summary, the present results demonstrate the appearance of LO products after plaque rupture and that increases in these products can occur in vivo with ASA therapy. They are also the first to identify putative products of transcellular eicosanoid metabolism in vivo. These results implicate the participation of atherosclerotic plaques or possible plaque-derived materials interacting with blood cells in the production of LO-derived eicosanoids. Taken together, they add to the complex repertoire of events that occurs after PTCA. Moreover, these findings suggest that pharmacological interventions both at the level of leukotriene inhibitors and receptor level antagonists may be useful in the prevention of PTCA-associated complications.

Note added in proof: Recent results from Salbach et al demonstrate that low density lipoproteins (LDL) can provide arachidonic acid for eicosanoid generation by activated monocytes in vitro. In view of our results, the conversion of LDL-derived arachidonic acid into lipoygenase products may also contribute to the mechanisms leading to leukotriene and lipoxin release after PTCA.

Acknowledgments

The authors thank Kelly Ann Sheppard for technical assistance and Helena Judge Li of the Channing Laboratories for technical supervision of the GC/MS. The authors also thank Dr. Robert L. Handin for critical reading of the manuscript, Dr. Samuel J. Shubrooks for additional sample acquisition, and Mary Halm Small for skillful preparation of the manuscript. We also thank Dr. Hugh Brady and Elizabeth VanDeCarr of the Renal Division for assistance.

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_Circulation_. 1992;86:56-63
doi: 10.1161/01.CIR.86.1.56

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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