Suppression of Eicosanoid Biosynthesis During Coronary Angioplasty by Fish Oil and Aspirin

Gregory A. Braden, MD; Howard R. Knapp, MD, PhD; and Garret A. FitzGerald, MD

Background. Percutaneous transluminal coronary angioplasty (PTCA) is an acute, localized stimulus to platelet and vascular function. Periprocedural cardiovascular complications are reduced by moderate-dose aspirin (ASA), presumably due to inhibition of thromboxane (TX) A₂.

Methods and Results. Excretion of TXA₂ and prostacyclin (PGI₂) metabolites in urine increased during PTCA. Pretreatment for 3 days with either moderate- (325 mg/day) or low-dose (80 mg/day) ASA inhibited the increase in both eicosanoids. Pretreatment for 3 weeks with fish oil (10 g/day) only partially suppressed TXA₂. Formation of trienoic eicosanoids and accumulation of ω-3 fatty acids in platelet membranes confirmed fish oil ingestion. Although basal PGI₂ was not inhibited, the PTCA-related increment was suppressed.

Conclusions. PTCA results in an acute, transient alteration of eicosanoid biosynthesis consistent with accelerated platelet–vascular interactions. Pretreatment for 3 days with moderate or low doses of ASA suppresses TXA to a similar extent during PTCA, and their effects on acute cardiovascular complications of this procedure are likely to be comparable. It is unlikely that even prolonged pretreatment with fish oil can substitute for the platelet inhibitory action of ASA during PTCA. Suppression of PGI₂ may contribute to the residual acute periprocedural complication rate in patients taking ASA. (Circulation 1991;84:679–685)

Although percutaneous coronary angioplasty (PTCA) is an effective approach to the relief of myocardial ischemia resulting from vascular occlusion, it is complicated by periprocedural myocardial infarction in roughly 5% of patients. Three months after the intervention, restenosis has occurred in an additional third. The vascular trauma caused by angioplasty is associated with increased platelet deposition on the dilated arterial segment, and moderate-dose (330 mg/day) aspirin therapy has been shown to reduce the incidence of periprocedural myocardial infarction by about 50%, presumably via inhibition of the synthesis of the platelet-derived eicosanoid thromboxane (TX) A₂. These observations and the role that activated platelets play as a substrate for accelerated conversion of the prothrombinase complex provide the basis for the use of antiplatelet and anticoagulant drugs as adjuvant therapy in PTCA. Although our understanding of the pharmacology of aspirin suggests that lower doses (less than 100 mg/day) might be as effective at suppressing TXA₂ formation as 330 mg/day, this has not been confirmed in patients undergoing PTCA. Such lower doses would be expected to have a reduced incidence of gastrointestinal side effects and to suppress formation of the platelet inhibitory eicosanoid prostacyclin (prostaglandin [PG] I₂) to a lesser extent.

Fish oils are rich in ω-3 fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid, which can substitute for the ω-6 fatty acid arachidonic acid (AA) in cell membranes. The incidence of death from ischemic heart disease has been inversely related to fish consumption in some epidemiological studies. This has been attributed in part to fish oil suppressing the vasoconstrictor, platelet agonist TXA₂ and leaving PGI₂ synthesis intact. Furthermore, while EPA-derived PGI₂ is as potent a platelet inhibitor and vasodilator as PGI₂, TXA₂ may be much less biologically active than TXA₂.

Although disputed, there is some evidence that feeding fish oil to patients before PTCA may reduce...
the incidence of restenosis,16,17 This may reflect inhibition of TXA-dependent platelet activation. However, this would be surprising, as aspirin does not appear to prevent restenosis4 and although very high doses of fish oil have modest inhibitory effects on chronic platelet activation in vivo in patients with severe peripheral vascular disease,18 they are of limited efficacy in an animal model of platelet-dependent coronary occlusion.19

To investigate the influence of fish oil and of both low and moderate doses of aspirin on eicosanoid biosynthesis during PTCA, we measured excretion of the major metabolites of TXA and PGI derived from both AA and EPA. This study provided the first opportunity to compare these effects in humans in the setting of an acute and potent stimulus to their formation and, in aspirin-sensitive patients, to determine the direct effect of such a localized intervention on these noninvasive indexes of platelet and vascular function.

Methods

The patients were recruited in the Nashville Veterans Administration (VA) Hospital and Vanderbilt University Hospital. All gave written consent before the procedure, and the study was approved by the committees for the protection of human subjects of both the VA and Vanderbilt University. All patients were men who were admitted for chest pain and had undergone elective coronary angiography at least 6 days before entry into the study. Their coronary anatomy was suitable for single-vessel PTCA; they appeared to be in a stable phase and were scheduled for elective PTCA before enrollment into the study. The majority of the patients were chronic smokers, although they abstained from cigarettes from 12 hours before PTCA to completion of the study. Antianginal medications were continued up to the time of PTCA (Table 1). PTCA was performed using a femoral approach. All patients received an intravenous bolus of 10,000 units heparin at the beginning of the procedure and hourly supplemental doses as required. Intracoronary nitroglycerin was administered as clinically indicated. The patients abstained from all antiplatelet drugs other than those involved in the study for at least 2 weeks before the investigation and until its completion.

Four groups of patients were studied. The first group (n=6) received fish oil, providing 10.0 g/day

<table>
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<th>Table 1. Patient Characteristics</th>
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<td><strong>Group</strong></td>
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<td>58</td>
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<td>54</td>
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<td>64</td>
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<tr>
<td>61±2</td>
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<td>80 mg aspirin</td>
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<td>68</td>
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<td>56±3</td>
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Vessel, vessel subjected to percutaneous transluminal coronary angioplasty; DM, type II diabetes mellitus; RCA, right coronary artery; Cx, circumflex coronary artery; LAD, left anterior descending coronary artery; C, calcium channel blocker; N, nitrates; B, β-blockers.
EPA and 6.8 g/day DHA in 60 ml MaxEPA (kindly provided by Dr. D. Davies, R.P. Scherer, Troy, Mich.). Aliquots were dispensed daily and patients were treated for at least 21 days before PTCA and for 7 days thereafter. Compliance was assessed by bottle counts and by measurement of platelet membrane fatty acids, as previously described.18 Two other groups of patients received 325 mg (n = 7) and 80 mg aspirin (n = 6) per day before the procedure for at least 3 days before and on the day of the procedure 4 hours before PTCA. Last, three patients with a documented history of aspirin sensitivity received no antiplatelet agents during the study period.

Urinary was collected in three successive 6-hour aliquots in all patients, commencing 6 hours before PTCA. Urine was analyzed for excretion of 2,3-dinor TXB2 (TXA2-M) and 2,3-dinor-6-keto PGF1α (PGF1-M) and their trienoic derivatives (TXA2-M and PGF1-M), major metabolites of TXA2/TXA3 and PGJ2/PGI3, respectively.16,20 Using gas chromatography–negative ion, chemical ionization–mass spectrometry as previously described.21,22 In fish oil–treated patients, blood was collected before and after 3 weeks of fish oil feeding and incubated for 45 minutes at 37°C. The serum was assessed for TXB2/TXB3 formation by gas chromatography–mass spectrometry.36

A nonparametric approach was employed to avoid assumptions regarding the distribution of the variables involved. Data were initially subject to analysis of variance by the Friedman test, followed by pairwise comparisons using the Mann–Whitney test as appropriate.23

**Results**

**Compliance**

Compliance with fish oil ingestion was confirmed with bottle counts, which suggested full consumption of the prescribed regimen. Assessment of platelet phospholipid fractions showed that there was substantial incorporation of both EPA and DHA at the expense of AA (Table 2). This was most marked in the phosphatidylinositol (PC) fraction in which the EPA/AA ratio rose from 0.01±0.004 in the pretreatment period to 0.29±0.06 (p<0.01) with fish oil administration, thus confirming significant consumption of the oils and their bioavailability for eicosanoid production.

**Effects on Serum TXB2**

Serum TXB2/TXB3, an index of the maximal capacity for platelet production of TXA2/TXA3 ex vivo (in contrast with TX metabolite excretion, which reflects actual TX synthesis in vivo24), was assessed in fish oil–treated patients before and after 3 weeks of fish oil consumption. This showed a fall in serum TXB2 from 296±85 ng/ml to 181±49 ng/ml, whereas serum TXB3 increased from 2.8±1.4 ng/ml to 12.9±2.7 ng/ml (p<0.05).

**Table 2. Fatty Acid Content of Platelet Membrane Phospholipid Before and After 3 Weeks of Fish Oil Feeding**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Fish oil fed</th>
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<tbody>
<tr>
<td>PC</td>
<td>AA</td>
<td>14.6±0.9</td>
</tr>
<tr>
<td></td>
<td>EPA</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td></td>
<td>DHA</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>PE</td>
<td>AA</td>
<td>39.6±1.5</td>
</tr>
<tr>
<td></td>
<td>EPA</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td></td>
<td>DHA</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>PS+PI</td>
<td>AA</td>
<td>26.2±0.7</td>
</tr>
<tr>
<td></td>
<td>EPA</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td></td>
<td>DHA</td>
<td>0.58±0.01</td>
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</tbody>
</table>

Arachidonic acid (AA) and the ω-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are expressed as percentage constituents of the phosphatidylinositol (PC), phosphatidylethanolamine (PE), and phosphatidylserine plus phosphatidylinositol fraction (PS+PI). Data are expressed as mean±SEM.

*p<0.05; †p<0.01.

**Effects on Basal Eicosanoid Biosynthesis**

Before angioplasty, TXA2-M excretion was 504±96 pg/mg creatinine in the aspirin-sensitive untreated control group, which is in the range previously reported in aspirin-sensitive patients with unstable angina during pain-free intervals.25 Pretreatment for 3 weeks with fish oil did not significantly depress the values below those observed in the control patients, although TXA2-M excretion was somewhat lower (418±129 pg/mg creatinine) (Figure 1). Excretion of total thromboxanes (TXA2-M and TXA3-M) tended to increase marginally from values before fish oil feeding in this group (Figure 2). As expected, excretion of TXA2-M in both aspirin-treated groups was markedly suppressed at 91±10 pg/mg creatinine for 325 mg aspirin and at 141±42 pg/mg creatinine for 80 mg aspirin (p<0.01) (Figure 1).

Fish oil pretreatment did not significantly depress basal PG12 biosynthesis from the values in the aspirin-sensitive patients (Figure 3), which again were in the

![Figure 1. Bar graph showing excretion of 2,3-dinor thromboxane (TX) B2 (TXA2-M) in successive 6-hour urinary aliquots commencing 6 hours before percutaneous transluminal coronary angioplasty. TXA2-M excretion increased significantly in aspirin-sensitive patients (open bars; p<0.01) and in those pretreated with fish oil (p<0.05). Pretreatment with aspirin (ASA) prevented the increase in TXA2-M during angioplasty. PG12, prostacyclin.](http://circ.ahajournals.org/resolve/doi/10.1161/01.CIR.0000124953.54621.7F)
Effects of 2,3-dinor TXA4 on Excretion of Prostaglandin (PG) I2-M

Excretion of TXA2-M and PGI2-M increased significantly (p<0.05) in the 6-hour collection corresponding to PTCA in the aspirin-sensitive patients, falling markedly in the subsequent collection (Figures 1 and 3). Similarly, increases in TXA2-M (p<0.05) and TXA2-M (p<0.04) were observed in the fish oil–treated patients (Figure 2). By contrast, the PTCA-associated increase in TXA2 biosynthesis was suppressed in both aspirin-treated groups (Figures 1 and 2).

Like TXA2-M excretion, there was a marked increase in PGI2-M excretion associated with PTCA in aspirin-sensitive patients. Fish oil pretreatment prevented the stimulated increase in both PGI2-M and PGI2-M during PTCA (Figure 2). Similarly, both doses of aspirin prevented the stimulated increase in PGI2 formation (Figure 3).

Discussion

Experimental evidence supports that platelet and vascular dysfunction accompanies PTCA3,26 and may contribute to its complications. We have confirmed that this occurs acutely in vivo by measurement of the excretion of major metabolites of the predominant eicosanoids formed by platelets and vascular endothelial–TXA2 and PGI2—in patients undergoing this procedure. Previous studies established the utility of this noninvasive approach in characterizing platelet–vascular interactions in vivo.24

We selected aspirin-sensitive patients as a control group, as ethical considerations determine that patients undergoing PTCA receive aspirin unless its use is contraindicated. We (H.R.K. and G.A.F.) have previously shown that TXA2-M and PGI2-M excretion in apparently healthy, asymptomatic aspirin-sensitive patients is similar to that in healthy volunteers. In the present study, we demonstrated that the levels of these metabolites excreted by our patients who had presented with angina but were sufficiently stable to permit elective PTCA were within the range that we have previously reported in patients with unstable angina who were free of pain.25

Aspirin, in a dose of 300 mg/day, has been shown to reduce the incidence of peri-procedural myocardial infarction in patients undergoing PTCA in a double-blind, placebo-controlled trial.4 By contrast, there was no apparent effect on the incidence of late restenosis, although the trial was too small to exclude a small benefit from aspirin.4 The biochemical pharmacology of aspirin27,28 and clinical trials performed since that time29,30 suggest that even lower doses would be at least as effective as inhibiting TxA2-dependent platelet activation. We assessed the effects of two doses of aspirin, 325 and 80 mg/day, having predosed the patients for a period (3 days) sufficient to achieve steady-state effects on platelet TXA2 formation.5,27 We found that the increase in TXA2 biosynthesis coincident with PTCA was short lived and that it was suppressed by pretreatment with both doses of aspirin. Thus, the lower dose might be expected to be at least as effective an inhibitor of TxA2-dependent platelet activation during PTCA as the more conventional therapy.
The theoretical attraction of using a lower dose of aspirin is twofold: a reduction in dose-related side effects and preservation of the ability of the vasculature to form PGl2, which may function as a limiting homeostatic response to platelet activation in vivo.\textsuperscript{31,32} In the present study, pretreatment with the higher dose of aspirin depressed PGl2 biosynthesis compared with the values observed in the control patients. This was not seen after pretreatment with low-dose aspirin, although a modest inhibitory effect could not be excluded due to the parallel nature of the trial design. However, during PTCA, a marked increase in PGl-M excretion occurred in the aspirin-sensitive controls but was suppressed in the patients who received both high- and low-dose aspirin. The increase in PGl2 biosynthesis during PTCA may reflect both mechanical trauma and physical and chemical stimulation due to platelet-vessel wall interactions.\textsuperscript{32} It is unclear at present whether maintenance of the ability to generate this eicosanoid during PTCA is of functional importance; however, infusion of ciprostene, a PGl2 analogue, reduces PTCA-related cardiovascular complications.\textsuperscript{33} The present study demonstrated that, even at low doses, chronic therapy with conventionally formulated aspirin is likely to suppress this response.

Fish oils have many pharmacological properties that may be relevant to the amelioration of cardiovascular disease.\textsuperscript{34} These include anti-inflammatory effects,\textsuperscript{35} the reduction of blood pressure,\textsuperscript{36,37} and the modulation of growth factor expression in endothelial cells.\textsuperscript{38} They have also been reported to inhibit platelet function,\textsuperscript{11,12} a property by which they might reduce the risk of thrombotic vascular disease. An explanation for the apparent relation between the bleeding tendency in Greenland Eskimos and their high dietary content of \(\omega-3\) fatty acids such as EPA in fatty fish and seal meat was provided by Dyerberg et al.\textsuperscript{11} Needleman et al\textsuperscript{13} reported that whereas AA-derived PGl2 and EPA-derived PGl were similarly effective as platelet inhibitors and vasodilators, TXA2 was more potent as a platelet agonist and vasoconstrictor than TXA3. In addition, PGH2 was metabolized to TXA3 much less efficiently in platelets than was its bisenoic analogue. Thus, a shift to an EPA-based diet at the expense of AA might be desirable in patients at risk of thrombosis. This hypothesis has been supported by the demonstration of an inverse relation between cardiovascular risk and fish consumption in epidemiological studies.\textsuperscript{8-10} although in some cases, the fish were a poor source of EPA\textsuperscript{8} and in other studies the relation was not observed.\textsuperscript{39,40}

There have been conflicting results concerning the efficacy of fish oil in preventing the restenosis that complicates PTCA.\textsuperscript{14,16,41,42} The present study was not designed to address this question. Clearly, eicosanoids are just one of many factors that might be relevant to the putative effect of fish oil in preventing restenosis,\textsuperscript{34} and a large-scale clinical trial is necessary to define the efficacy and safety of this type of intervention in PTCA. However, given the likely relevance of TXA2-dependent platelet activation to the periprocedural ischemic events that complicate PTCA,\textsuperscript{4} it is of importance to define the extent to which fish oil modulates TX formation during this procedure.

Previous studies in healthy volunteers suggest that several weeks of pretreatment are necessary to maximize the effects of fish oil on platelet and vascular function,\textsuperscript{42-44} and a large prospective study of fish oil in PTCA has been initiated with a 3-week pretreatment period.\textsuperscript{41} If such a requirement were necessary to observe clinical benefit from fish oil, its use would be restricted to patients undergoing elective PTCA, and it would place considerable demands upon patient compliance.

We wished to assess the practicality of such a regimen in PTCA patients by comparison with our previous experience of feeding fish oil to highly motivated, healthy volunteers who knew that they were subject to daily assessment and biochemical monitoring.\textsuperscript{18} To maximize the likelihood of a detectable effect on eicosanoid formation, we administered a dose of fish oil that contained an amount of EPA roughly equivalent to that reported in the Eskimo diet\textsuperscript{18} and selected a patient population similar to that reported by Dehmer et al\textsuperscript{16} in the study that supported the efficacy of fish oil in preventing restenosis.

Fish oil consumption by our patients was confirmed by formation of trienoic metabolites of TX and PGl and by the accumulation of EPA at the expense of AA in platelet membrane phospholipids. However, both indexes suggest that compliance was less than we have previously observed in the more compulsively scrutinized healthy volunteers who received the same dosing regimen. Thus, whereas the EPA/AA ratio rose from a mean 0.01 to 0.29 in the platelet PC in the current study, it rose from 0.04 to 0.76 in the healthy volunteers. The magnitude of trienoic metabolite formation was also more impressive in the latter group.\textsuperscript{18} These results are not surprising, given the unpalatability of fish oils and the limited success obtained in the only study in which compliance was assessed quantitatively (by capsule count) in PTCA patients who were given a lower dosage for a shorter period of time.\textsuperscript{14} Consistent with these results, serum TXB2 fell a mean 39% in the present study. This modest decline would be likely to result in a minor impairment in TXA-dependent platelet activation\textsuperscript{43} and is consistent with the failure to detect a fall in the excretion of TXA2-M, which, as we have previously noted, requires consumption of large doses of fish oil.\textsuperscript{18}

Pretreatment with fish oil only partially suppressed the increase in TXA2 formation associated with PTCA. It did not suppress PGl2 formation before PTCA. By contrast, the increase in PGl2 biosynthesis observed in the control group during PTCA did not occur in the patients fed fish oil, nor was it compensated for by the rise in PGl3. These results are surprising, based on the failure of fish oil to suppress PGl2 formation in volunteers. However, they are
consistent with the effects of fish oil feeding in an experimental model of platelet activation in the coronary circulation, after thrombolyis with tissue plasminogen activator in the dog.19 In this setting, a marked increase in TX-M and PGI-M excretion accompanies platelet deposition at the site of the lysed thrombus and results ultimately in coronary occlusion.46 Chronic administration of high-dose fish oil caused a partial but incomplete suppression of this increment in TXA2-M excretion, whereas the lysis-associated increase in PGI2 formation was completely suppressed. The antithrombotic effect of fish oil in this setting is correspondingly limited.19 In summary, it appears that fish oil supplementation does not suppress basal formation of PGI2 but rather inhibits the increment in PGI2 formation that is observed in syndromes of platelet activation and vascular injury. Whether this limits the clinical efficacy of fish oil in preventing vascular occlusive disease is unknown.

The present investigation establishes that an acute, localized intervention in the coronary vasculature results in an increase in urinary TX and PGI metabolites, noninvasive indexes of platelet and vascular function. These changes are transient, rendering their contribution (if any) to long-term complications like restenosis unclear. We have shown that even chronic administration of high doses of fish oil fails to suppress the increase in TXA2 formation that accompanies PTCA. We have demonstrated that moderate-dose aspirin suppresses TXA2 during angioplasty, which is consistent with its ability to reduce the incidence of periprocedural myocardial infarction. Our biochemical studies suggest that a lower dose (e.g., 80 mg/day for 3 days) of aspirin, but not the fish oil regimen that we used, is likely to be similarly effective. Surprisingly, both fish oil and low-dose aspirin, as well as moderate-dose aspirin, suppress the increase in PGI2 synthesis during PTCA. Inhibition of PGI2 may be relevant to the residual frequency of acute thrombotic complications of PTCA in patients taking conventional formulations of aspirin.

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KEY WORDS • ω-3 fatty acids • percutaneous transluminal coronary angioplasty • aspirin
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