Effects of Cyclooxygenases Inhibitors on Vasoactive Prostanoids and Thrombin Generation at the Site of Microvascular Injury in Healthy Men

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Objective—Balance between vasoactive prostanoids that contribute to homeostasis of the circulatory system can be affected by cyclooxygenases inhibitors. Results of a recent large clinical trial show that myocardial infarction was more frequent among patients with rheumatoid arthritis treated with the selective cyclooxygenase-2 inhibitor rofecoxib compared with those treated with naproxen. Whether this difference was attributable to deleterious cardiovascular effects of rofecoxib or cardioprotective effects of naproxen has not been determined. We tested the hypothesis that naproxen, contrary to rofecoxib, exerts antithrombotic effects.

Methods and Results—Forty-five healthy men were randomized to receive a 7-day treatment with rofecoxib (50 mg/d), naproxen (1000 mg/d), aspirin (75 mg/d), or diclofenac (150 mg/d). Formation of thromboxane, prostacyclin, and thrombin in the bleeding-time blood at the site of standardized microvascular injury was assessed before and after treatment. Naproxen, like aspirin, caused significant reduction of both thromboxane and prostacyclin, whereas diclofenac depressed prostacyclin synthesis but had no effect on thromboxane formation. Naproxen and aspirin significantly suppressed thrombin generation. Diclofenac showed a similar tendency, which did not reach statistical significance. Rofecoxib had no effect on any variables measured.

Conclusions—In healthy men, naproxen exerts an antithrombotic effect at least as potent as aspirin, whereas rofecoxib does not affect hemostatic balance. (Arterioscler Thromb Vasc Biol. 2003;23:1111-1115.)

Key Words: myocardial infarction ■ risk factors ■ cyclooxygenase inhibitors ■ prostaglandins ■ thrombin
activity in platelets. The injured endothelium and flowing blood might correspond to the platelet-wall interactions going on during the atherosclerotic plaque formation or rupture, although the flow is laminar rather than turbulent and muscle cells are absent.

Methods

Design of the Study

In this double-blind study, healthy men were randomly allocated into 4 treatment groups. Each group was treated for 7 consecutive days with 1 of the following NSAIDs: rofecoxib (25 mg twice daily; n=18), naproxen (500 mg twice daily; n=9), aspirin (75 mg daily; n=9), or diclofenac (75 mg twice daily; n=9). Rofecoxib and naproxen doses corresponded to those used in the VIGOR study, diclofenac was given in the most often prescribed dose, and aspirin was given in the minimal therapeutic dose sufficient for platelet function inhibition. The skin-bleeding time with blood collection was performed on the first day, before the drug administration, and was repeated on the 7th day, 4 to 6 hours after the morning drug dose.

Subjects

Participants of the study were recruited from symptom-free, non-smoking, healthy volunteers, aged 20 to 30 years (mean, 23 years), who did not take any drug for at least 2 weeks. Arachidonic acid at a concentration of 900 μmol/L produced platelet aggregation in their platelet-rich plasma. Forty-five men who completed the study had no personal history of gastrointestinal disease, drug allergy, thrombotic disorders, or bleeding disorders. The protocol was approved by the University Ethics Committee, and all subjects gave informed consent.

Model of Microvascular Injury

The eicosanoids studied and the tissue factor–initiated coagulation were evaluated in samples of bleeding-time blood, as described previously. Briefly, after compressing the upper arm with a sphygmomanometer cuff to 40 mm Hg, 2 standardized incisions were made on the forearm skin with use of a Simplet II device (Organon Teknika). The procedure was always performed by the same investigator. The blood shed was collected at 30 seconds and then at 1-minute intervals directly from the edge of the skin wound into microcuvettes and then passed into Eppendorf tubes containing an anticoagulant mixture composed of 100 mmol EDTA and 60 μmol/L indomethacin in 0.9% NaCl. The tubes were centrifuged, and the supernates were removed, aliquoted, and stored at −80°C for additional analysis.

TXB₂ and 6-keto-PGF₁α were determined using a RIA Amersham assay. Thrombin-antithrombin complexes, reflecting thrombin generation, were determined by ELISA (Enzygnost TAT Micro, Dade) and expressed as nanomolar concentrations, whereas the eicosanoids concentrations were expressed as picograms per milliliter of oozing blood. When 150 mg indomethacin per 24 hours was given to healthy men aged 20 to 25 years, the levels of 6-keto-PGF₁α (25 mg twice daily; n=18), naproxen (500 mg twice daily; n=9), aspirin (75 mg daily; n=9), or diclofenac (75 mg twice daily; n=9). Rofecoxib and naproxen doses corresponded to those used in the VIGOR study, diclofenac was given in the most often prescribed dose, and aspirin was given in the minimal therapeutic dose sufficient for platelet function inhibition. The skin-bleeding time with blood collection was performed on the first day, before the drug administration, and was repeated on the 7th day, 4 to 6 hours after the morning drug dose.

Results

Aspirin, naproxen, and diclofenac caused statistically significant increase in bleeding time (Figure 1; available online at http://atvb.ahajournals.org) and in the volume of blood oozing from the bleeding time incisions. Platelet aggregation in response to arachidonic acid became inhibited. Rofecoxib had no effect on bleeding time, volume of blood, or platelet aggregation.

The median values of 6-keto-PGF₁α for 3 NSAIDs recorded at 270 seconds were 119 pg/mL (1,3 quartile, 78; 138 pg/mL) before and 46 pg/mL (1,3 quartile, 39; 52 pg/mL) after the treatment, whereas for rofecoxib, they were 130 pg/mL (1,3 quartile, 87; 139 pg/mL) and 100 pg/mL (1,3 quartile, 79; 151 pg/mL), respectively. Similar data for TXB₂ were 2956 pg/mL (1,3 quartile, 1581; 5476 pg/mL) and 137 pg/mL (1,3 quartile, 90; 1304 pg/mL) for NSAIDs and 3012 (1,3 quartile, 2120; 4219 pg/mL) and 2432 pg/mL (1,3 quartile, 1618; 2993 pg/mL) for rofecoxib. In case of TAT, the median values obtained at the same time of blood sampling were 42 nmol/L (1,3 quartile, 26; 59 nmol/L) before NSAIDs and 20 nmol/L (1,3 quartile, 14; 26 nmol/L) after 7-day therapy, and 17 nmol/L (1,3 quartile, 14; 28 nmol/L) and 16 nmol/L (1,3 quartile, 12; 18 nmol/L) for rofecoxib, respectively.

Aspirin blocked TXB₂ and 6-keto-PGF₁α formation in clotting blood (Figure 1). Naproxen significantly decreased both 6-keto-PGF₁α and TXB₂ levels. Diclofenac displayed a weak trend toward diminution of TXB₂ and depressed 6-keto-PGF₁α levels. In the rofecoxib group, the levels of both PGI₂ and TXA₂ metabolites were almost identical before and after 7 days of treatment. Because balance between the 2 eicosanoids studied maintains cardiovascular homeostasis, we calculated their ratio at 60-second intervals (Figure 2). Both aspirin and naproxen shifted the ratio toward PGI₂ metabolite, whereas rofecoxib and diclofenac had no such effect. Aspirin and naproxen produced a significant fall in thrombin generation. Diclofenac showed similar tendency, which did not reach statistical significance. Rofecoxib did not affect thrombin generation (Figure 4, available online at http://atvb.ahajournals.org).

Discussion

From the cardiovascular standpoint, the safety of COX-2 inhibitors has been the topic of much discussion and controversy. Concerns have been raised that selective blockade of COX-2 may impair endothelial function and promote cardiovascular disease, especially when coxibs are used at a high dose or aspirin is not given concomitantly in patients who meet criteria for its use as a preventive agent. These views seem to be supported by a recent experimental study. Smooth muscle cell proliferation in response to carotid artery endothelial injury was increased in mice lacking the prostacyclin receptor and decreased in mice lacking the thromboxane A₂ receptor. However, prostacyclin receptor–deficient mice, which represent the complete elimination of prostacyclin receptor–mediated biologic effects of PGI₂, may not reflect
the effects of selective COX-2 inhibition,\textsuperscript{21} because COX-1 also contributes to endothelial cell synthesis of PGI\textsubscript{2}.\textsuperscript{22,23} On the other hand, coxibs may have a beneficial effect on cardiovascular events in atherothrombosis.\textsuperscript{24,25} COX-2 expression is upregulated in atherosclerotic plaques. Proinflammatory cytokines and growth factors enhance COX-2 expression in macrophages and monocytes in atherosclerotic plaque proximity.\textsuperscript{23} This leads to excessive production of proinflammatory eicosanoids (PGE\textsubscript{2}, PGD\textsubscript{2}, and TXA\textsubscript{2})\textsuperscript{26} and metalloproteinases.\textsuperscript{21,27} In parallel, the exaggerated expression of COX-2 observed in the endothelial cells and smooth muscles results in the upregulation of endothelial NO synthase and enhanced NO production.\textsuperscript{28} COX-2 inhibition may decrease vascular inflammation and improve plaque stability.\textsuperscript{26} A recent pilot study indicated that in patients with acute coronary syndrome without ST-segment elevation, treatment with a selective COX-2 inhibitor, meloxicam, together with heparin and aspirin was associated with a significant reduction in adverse outcomes compared with treatment with heparin and aspirin alone.\textsuperscript{29} Studies in the mouse have clearly shown\textsuperscript{30} that the inhibition of COX-2 or the deletion of the COX-2 gene in the arterial macrophage reduces atherosclerosis.

The drugs we studied have decreasing ability to inhibit COX-1 in vitro,\textsuperscript{1} with aspirin producing full inhibition of both isoforms, followed by naproxen and then diclofenac, with moderate preference toward COX-2 and rofecoxib, a highly selective COX-2 inhibitor. Because COX-1 in platelets is the major source of TXA\textsubscript{2}, it is not surprising that both aspirin and naproxen strongly inhibited its formation whereas the effects of diclofenac were less accentuated and rofecoxib was deprived of such activity. The drugs we investigated in a...
parallel fashion affected formation of prostacyclin, with aspirin being the strongest inhibitor and rofecoxib having no inhibitory activity. Similar to other authors,10,11 we expressed eicosanoid concentrations in picograms or nanograms per milliliter of blood; the conclusions of the study remained the same if data were expressed in picograms per second. Importantly, both naproxen and aspirin shifted the PGI₂/TXA₄ ratio in favor of prostacyclin. Naproxen was shown to be a potent and long-lasting (up to 8 hours) COX-1 inhibitor, which explains its antiplatelet efficacy.32 The drug only slightly decreased prostacyclin production, resulting in favorable balance of PGI₂/TXA₄. Diclofenac prolonged bleeding time and decreased prostacyclin generation but had no effect on thrombin generation and thromboxane levels. Rofecoxib did not affect any parameter measured. These results differentiate clearly the above 2 drugs from aspirin and naproxen, characterized by marked COX-1 selectivity. Our results indicate that coxibs do not necessarily lead to depressed prostacyclin production. This is in contrast to the conclusions of McAdam et al4 and Catella-Lawson et al,33 based on decreased excretion of urinary prostacyclin metabolite in healthy volunteers treated with celecoxib.

The population in this study was healthy and free of known cardiovascular risk factors. The results, therefore, should not be extrapolated leniently to patients with atherosclerosis, in whom prostanooids are known to play an important role in homeostasis of cardiovascular system.

Our results point to powerful antithrombotic effects of naproxen. They provide biochemical evidence supporting 3 recent case-control studies34–36 that demonstrate that patients treated with naproxen have a decreased incidence of myocardial infarction compared with patients receiving NSAIDs other than naproxen or those not receiving NSAIDs. Our data also corroborate observations37 that showed that neither rofecoxib nor naproxen, when used at the therapeutic doses, impairs endothelial vascular response in healthy volunteers. Finally, our results indicate that in human microvasculature, COX-1 and not COX-2 seems to be the source of prostacyclin.

Acknowledgments
This work was supported by research grants from the Polish State Research Council, Warsaw and MSD, Warsaw, Poland.

References

Figure 2. Effects of the four drugs studied on the ratio of 6-keto-PGF₁α to TXB₂ (ordinate). Abscissa time of blood sampling (seconds). n=18 for rofecoxib; n=9 for the remaining 3 drugs studied.


20. Tuleja et al. Coxibs and NSAIDs in Atherosclerosis.
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Arterioscler Thromb Vasc Biol. 2003;23:1111-1115; originally published online May 1, 2003; doi: 10.1161/01.ATV.0000074879.19006.51

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/23/6/1111

Data Supplement (unedited) at:
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A

Mean and 95% CI

PGFα [pg/ml]

Time

Diclofenac

Naproxen

Aspirin

Rofecoxib

p=0.009

p=0.047

p<0.001

p=0.565

B

Mean and 95% CI

Log [Xβ]

Time

Diclofenac

Naproxen

Aspirin

Rofecoxib

p=0.180

p<0.001

p<0.001

p=0.157

[Diagram showing the mean and 95% confidence interval for PGFα and Log [Xβ] over time for Diclofenac, Naproxen, Aspirin, and Rofecoxib, with statistical p-values for each comparison.]