Improved Endothelial Function by the Thromboxane A2 Receptor Antagonist S 18886 in Patients With Coronary Artery Disease Treated With Aspirin

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OBJECTIVES
In this study, we evaluated the effect of S 18886, a specific thromboxane A2 receptor antagonist, on endothelial function in patients with coronary artery disease (CAD).

BACKGROUND
Impaired release of endothelial vasodilator substances and increased release of vasoconstrictor prostanoids both contribute to endothelial dysfunction in atherosclerosis. One unresolved question is whether vasoconstrictor prostanoids are still produced and affect vascular tone or alter endothelium-dependent vasodilation in patients treated with aspirin.

METHODS
Twenty patients with stable CAD treated with 100 mg/day aspirin were evaluated in a randomized, double-blinded, placebo-controlled study. Twelve patients received a single oral dose of 10 mg S 18886, and eight patients received placebo. Before and 60 min after a single oral dose of S 18886 or placebo, flow-mediated vasodilation (FMD) was evaluated using an echo-tracking device. Venous occlusion plethysmography was used to evaluate the effects on forearm blood flow (FBF) of a brachial artery infusion of acetylcholine (ACh), sodium nitroprusside (SNP), or norepinephrine before and after treatment.

RESULTS
Baseline FBF was not affected by S 18886 or placebo. The vasodilator response to ACh was significantly potentiated by S 18886 as compared with placebo (p = 0.03 by analysis of co-variance), whereas the effects of norepinephrine and SNP were unchanged. Flow-mediated dilation increased from 2.50 ± 1.14% to 3.84 ± 1.80% (p < 0.01) after S 18886, but was unchanged after placebo.

CONCLUSIONS
Single administration of S 18886 improved FMD and ACh-induced vasodilation in aspirin-treated patients with CAD. These results suggest that release of endogenous agonists of TP receptors may contribute to endothelial dysfunction, despite aspirin treatment, in patients with atherosclerosis. (J Am Coll Cardiol 2003;41:1198–204) © 2003 by the American College of Cardiology Foundation

Impaired endothelium-dependent relaxation is a major characteristic of the diseased vessel wall (1,2). Endothelial dysfunction stems chiefly from an inability of the endothelial cell to release vasodilating substances such as nitric oxide, prostacyclin, or endothelin-derived hyperpolarizing factor (3). A current hypothesis is that impairment of endothelium-dependent relaxation in atherosclerosis may be paralleled by a propensity to release endothelium-contracting factors, such as thromboxane A2 (TXA2), superoxide anions, and the peptide endothelin (4).

One consequence of endothelial dysfunction is enhancement of platelet-endothelial cell interactions responsible for increased production of TXA2. Thromboxane A2 promotes aggregation, vasoconstriction, and proliferation by docking with a membrane-bound receptor, the TP receptor. The TP receptors can bind to other vasoconstrictor prostanoids, such as prostaglandin F2α (PGF2α) and the PGF2-like compounds isoprostanes (5). Isoprostanes are nonenzymatic products from cell membrane phospholipids and are released in response to oxidative stress (6) in disease states such as hypercholesterolemia (7,8), diabetes mellitus (9), and unstable angina (10). Aspirin, which is effective in reducing the risk of stroke and myocardial infarction, does not inhibit isoprostane formation. Moreover, TXA2 formation may be only partly inhibited by aspirin under certain pathologic conditions. Thus, an important question is whether vasoconstrictor prostanoids still contribute to endothelial dysfunction and affect vascular tone, even in patients treated with aspirin.

Because a TP-receptor antagonist may inhibit the effects of both TXA2 and isoprostanes, we designed the present study to investigate whether S 18886, a selective TP-receptor antagonist (11,12), could affect vascular tone and/or improve endothelial function in patients receiving low-dose aspirin to treat coronary artery disease (CAD). We used a double-blinded, randomized, parallel, placebo-controlled design to assess the effect of a single oral dose of S 18886 on forearm blood flow (FBF) variations in 20 CAD patients taking 100 mg/day aspirin. Endothelium-dependent vasodilation was examined by recording brachial
The FBF determinations by VOP were repeated 60 min after the S 18886 or placebo dose. The brachial artery catheter was removed, and FMD was measured in the contralateral brachial artery.

**Evaluation of FMD.** The FMD measurements were performed as previously described (14). All measurements were performed after a 30-min rest in bed, in a temperature-controlled room (22°C), with continuous blood pressure monitoring (Finapres 2300, Ohmeda, Englewood, Colorado). A high-resolution ultrasound Wall Track system (Pie Medical, Maastricht, the Netherlands) with a 7-MHz linear probe was used to measure the systolic and diastolic internal diameters of the distal brachial artery. This echo-tracking system, which analyzes radiofrequency signals, has a precision for diastolic diameter measurements of 30 μm. The FMD was measured following the increase in the brachial artery diastolic diameter after 3 min of ischemia of the ipsilateral hand, induced using a wrist cuff inflated at 200 mm Hg (hyperemia test). When the wrist cuff is deflated, blood flow and shear stress increase briefly, inducing endothelial nitric oxide release and FMD. The maximum diameter (Dₘₐₓ) was defined as the greatest diastolic diameter following deflation of the cuff; measurements were made at deflation over five or six cardiac cycles and every 30 s thereafter for 5 min. The measurement at deflation was the minimum diameter (Dₜₐₚ) for basal diameter). The FMD (%) was calculated as: 100 × (Dₘₐₓ − Dₜₐₚ)/Dₜₐₚ.

**Measurement of FBF by VOP.** The VOP measurements were performed as previously described (15). Briefly, a mercury-in-silastic strain gauge was placed around the forearm. The strain gauge was electrically coupled to a calibrated plethysmograph (Perivein, JSI, ETNA, Noisy Le Grand, France). For each measurement, venous flow was occluded just proximal to the elbow by rapidly inflating a blood pressure cuff to 40 mm Hg. A wrist cuff was inflated to suprasystolic pressures starting 1 min before each measurement to exclude the hand circulation from blood flow determination. The FBF measurements are reported in ml/min per 100 ml of forearm volume, and each value is the mean value of at least three determinations. Systolic blood pressure, diastolic blood pressure, mean blood pressure, and heart rate were monitored continuously (Finapres 2300, Ohmeda). All studies were performed in the morning, in a quiet room kept at a controlled temperature of 22°C. While the subject was in the supine position, a catheter was inserted after local anesthesia (2% xylocaine) into the brachial artery of the nondominant arm, which was elevated to a level slightly above the right atrium. To establish rest control FBF values, 5% dextrose in water was administered for 30 min, and blood flow measurements were then repeated until a stable baseline condition was obtained. Infusion of vasoactive agents was then started. Between each series of drug injections, FBF was allowed to return to its basal value. During this period, 5% dextrose in water was infused. Three drugs were used to explore endothelial function: 1) ACh (Pharmacie Centrale des Hôpitaux, Paris,
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>S 18886 Group</th>
<th>Placebo Group</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>59.4 ± 6.2</td>
<td>58.4 ± 8.4</td>
<td>NS</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>25.5 ± 3.0</td>
<td>26.0 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>59.3 ± 6.6</td>
<td>54.8 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>130.0 ± 13.8</td>
<td>128.8 ± 11.3</td>
<td>NS</td>
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<td>(mm Hg)</td>
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<tr>
<td>Diastolic blood pressure</td>
<td>70.0 ± 10.7</td>
<td>67.9 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
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<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.6 ± 1.2</td>
<td>4.8 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.6 ± 1.0</td>
<td>2.9 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>5.7 ± 0.5</td>
<td>6.3 ± 1.8</td>
<td>NS</td>
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<td>Treatment with* (n)</td>
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<tr>
<td>Beta-blockers</td>
<td>10</td>
<td>8</td>
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<td>HMG CoA reductase inhibitors</td>
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<tr>
<td>Calcium channel blockers</td>
<td>8</td>
<td>3</td>
<td>NS</td>
</tr>
</tbody>
</table>

*All patients were treated by aspirin 100 mg/day.

ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; HMG CoA = hydroxymethyl glutaryl coenzyme A; LDL = low-density lipoprotein; NS = not significant.

France), as a continuous infusion at rates of 20, 40, and 80 μg/min; 2) SNP (Nitriate, Laboratoires SERB, Paris, France), at rates of 0.5 and 1 μg/min; and 3) norepinephrine (Pharmacie Centrale des Hôpitaux) at rates of 100, 500, and 1000 pmol/min.

Safety measures. Bleeding-time measurements were obtained at selection and 2 h after treatment with either S 18886 or placebo. Bleeding-time measurements were performed according to the Ivy Nelson method, on the forearm, with a Simplate device (Organon Technika, Eppelheim, Germany) (16). Physical examinations, cardiovascular parameters (supine blood pressure, heart rate, and electrocardiography), blood and urine biochemical parameters, hematology, and coagulation tests were conducted throughout the study.

Statistical analysis. All data are reported as the mean value ± SD. The results of VOP are expressed, for each patient, as a continuous infusion at rates of 20, 40, and 80 μg/min; 2) SNP (Nitriate, Laboratoires SERB, Paris, France), at rates of 0.5 and 1 μg/min; and 3) norepinephrine (Pharmacie Centrale des Hôpitaux) at rates of 100, 500, and 1000 pmol/min.

RESULTS

Study population. The general characteristics of the assessed patients are shown in Table 1. Age, hemodynamic parameters, and risk factors did not differ between the S 18886 and placebo groups. Most patients (90%) were receiving beta-blocking therapy at the time of the study. The groups did not significantly differ with respect to previous treatment with vasodilators and hydroxymethyl glutaryl coenzyme A reductase inhibitors. Bleeding time measured at baseline in patients already treated with 100 mg/day aspirin was similar in the S 18886 and placebo groups (5.45 ± 2.5 vs. 4.9 ± 1.5 min, respectively) and remained unchanged after treatment (5.1 ± 1.2 vs. 4.3 ± 1.3 min). No clinically relevant biochemical, hemologic, or coagulation abnormalities or changes in cardiovascular parameters possibly related to drug administration were detected. No serious adverse events were reported.

Brachial artery FMD. Brachial artery diameters recorded at baseline in the S 18886 and placebo groups were 5.48 ± 0.47 and 5.32 ± 0.34 mm, respectively, during the control phase and remained unchanged (5.24 ± 0.56 and 5.17 ± 0.59 mm) after treatment. Brachial artery FMD also did not differ at baseline between the S 18886 and placebo groups (2.50 ± 1.14% vs. 2.46 ± 0.76%, respectively; p = NS). After administration of S 18886, brachial artery FMD increased by >50% (2.50 ± 1.14% to 3.84 ± 1.80%, p = 0.01), whereas it remained unchanged after placebo (2.46 ± 0.76% to 3.01 ± 1.30%, p = NS) (Fig. 1).

Measurements of FBF using VOP. The baseline measurements did not differ between the S 18886 and placebo groups (Fig. 2). The mean baseline FBF values were 1.9 ± 0.8 and 2.0 ± 0.5 ml/min per 100 ml in the S 18886 and placebo groups, respectively (Fig. 2). The increases in FBF (expressed as the mean AUC arbitrary units) were 499.8 ± 259.6 with ACh and 3.78 ± 4.10 ± 0.69 with SNP for the S 18886 and placebo groups, respectively. The decreases in FBF with norepinephrine were similar in the S 18886 group (1255.9 ± 558.2) and placebo group (1548.1 ± 469.5).

Neither S 18886 nor placebo altered the baseline FBF values (Fig. 2). The vasodilator response to ACh, which significantly increased after treatment with S 18886, remained unchanged after dosing with placebo. As shown in Figure 2, the ACh-induced FBF increase in the S 18886 group (expressed as mean AUC) was larger after dosing...
than before treatment (849.3 ± 590.0 vs. 499.8 ± 265.2, respectively; p = 0.02). Comparisons of values recorded after treatment showed that the FBF response to ACh was significantly more increased in the S 18886 group than in the placebo group (849.3 ± 590.0 vs. 508.7 ± 293.9, respectively; p = 0.03 by ANCOVA). In contrast to ACh-induced vasodilation, SNP-induced vasodilation and norepinephrine-induced vasoconstriction were unaffected by S 18886 treatment (ANCOVA) (Fig. 3 and 4).

**DISCUSSION**

In this double-blinded, randomized, placebo-controlled study, we showed that a single 10-mg oral dose of S 18886, a new TP-receptor antagonist, significantly improved endothelium-dependent vasodilation in the peripheral arteries of patients with CAD treated with aspirin. There was an improvement in both ACh- and flow-mediated vasodilation. In addition, S 18886 neither affected baseline forearm vascular tone nor altered FBF responses to brachial artery infusion of SNP or norepinephrine.

**Vasoconstrictor prostanoids are involved in the control of endothelial function.** Aspirin is the most widely used prophylactic treatment for acute thrombotic complications of atherosclerotic cardiovascular disease. Its therapeutic effect is widely attributed to its ability to inhibit cyclooxygenase and, thus, the production of TXA2, which promotes platelet aggregation, vasoconstriction, and cell proliferation. Aspirin is usually given at low dosages that are expected to selectively inhibit platelet TXA2 synthesis. However, recent studies have suggested that aspirin may improve endothelial function in atherosclerosis when infused in high concentrations (18,19). These findings led to the hypothesis that aspirin may inhibit not only platelet TXA2 synthesis, but also formation of constricting factor synthesized in response to endothelial cell stimulation.

Acetylcholine stimulation of endothelial vasoconstrictor
prostanoid release has been well documented in previous experimental studies (20,21). Prostanoids have been implicated in endothelial dysfunction in hypertension (21,22), heart failure (23,24), and atherosclerosis (18). In these pathologic states, the defective response to ACh or shear has been widely ascribed to an imbalance between the release of endothelium-dependent relaxing factors and vasoconstricting factors.

Recent studies suggest that “aspirin-insensitive” vasoconstrictor prostanoids, such as isoprostanes, may be synthesized by endothelial cells in atherosclerotic cardiovascular diseases (10). Superoxide anion production has been shown in response to ACh in canine basilar artery endothelial cells, as well as shear stress in human umbilical endothelial cells (25–27). Therefore, ACh and shear stress may potentially lead to increased formation of isoprostanes, which are not blocked by aspirin treatment.

TP-receptor blockade improves endothelial function. In the present study of patients with documented CAD, we found that TP-receptor blockade improved both ACh- and shear stress–stimulated endothelium-dependent vasodilation. Treatment with S 18886 neither altered baseline forearm blood flow nor affected the responses to SNP or norepinephrine infusion, suggesting that it selectively increased endothelium-dependent vasodilation. Thus, our results strongly suggest that in patients with atherosclerosis, vasoconstrictor prostanoids acting on TP receptors are actively produced and released in response to endothelial stimulation by either ACh or shear stress. In keeping with standard clinical practice, all patients were treated with low-dose oral aspirin, which is known to inhibit platelet TXA2 production (28). As expected, we found that arachidonic acid–induced platelet aggregation was abolished in patients treated with this dose of aspirin. It is therefore unlikely that the effect of TP-receptor blockade observed in our patients was related to inhibition of residual platelet TXA2 formation. However, we cannot exclude that systemic endothelial or other vascular cells remain capable of producing TXA2 through a transcellular mechanism involving a functional cyclooxygenase pathway. Other endogenous TP-receptor agonists that are likely to play a role in patients with CAD include isoprostanes, whose production is probably increased in situations of cell dysfunction. The observation that a higher dose of aspirin improves endothelium-dependent vasodilation in patients with atherosclerosis does not conflict with this hypothesis, given that high concentrations of aspirin are known to exhibit antioxidant properties (30). Whatever the type of vasoconstrictor prostanoids involved in these responses, our results clearly indicate that TP-receptor blockade is more powerful than aspirin in limiting the deleterious effects of constrictive prostanoids in atherosclerosis. In theory, a TP-receptor antagonist may also offer the advantage of not interfering with the synthesis of vasodilator prostanoids. In Figure 5, we propose a mechanism to explain the beneficial effect of S 18886 on endothelial function.

These results are also consistent with a recent report by Cayatte et al. (31), in which S 18886 delayed atherogenesis in apolipoprotein E–deficient knockout mice. Interestingly, aspirin had no effect in this study, although it abolished TXA2 formation. It could be concluded that TP-receptor blockade inhibited atherosclerosis by a mechanism independent of platelet-derived TXA2. Together with our results, these findings suggest that TP-receptor blockade may offer greater vascular protection than aspirin. Conclusions. The present results also provide additional support for the possibility that the impairment of endothelium-dependent relaxation seen in patients with atherosclerosis may be paralleled by a propensity to release TP-receptor agonists. The precise nature of these agonists and the mechanism involved in their production still need to be elucidated. This may partly explain the resistance to aspirin, which is associated with an increased risk of
myocardial infarction and cardiovascular death (32). This suggests that TP antagonists may be candidates in further trials to evaluate their potential benefits in atherosclerosis.

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REFERENCES


