Thromboxane Antagonism and Endothelial Function

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
artery diameter variations in response to hyperemia and by using venous occlusion plethysmography (VOP) to measure the FBF response to a brachial artery infusion of acetylcholine (ACh).

**METHODS**

**Patient population.** We assessed endothelial function in 20 male patients with a mean (±SD) age of 59 ± 7 years (range 49 to 69). All had stable CAD documented by a previous coronary angiogram, with >30% stenosis in at least one site on a major branch; endothelial dysfunction manifesting as impaired flow-mediated dilation (FMD < 4.1%) (13,14); and a bleeding time < 12 min. None of the patients had a history of recent myocardial infarction, heart failure, cardiac arrhythmias, or uncontrolled hypertension. Patients with a history of hemostatic disorder, allergy, diabetes, or heavy smoking (>10 cigarettes/day) were not included in the study. Vasodilators and antihypertensive drugs were withdrawn at least 48 h before inclusion (except for beta-blockers, which were maintained throughout the study period for ethical reasons). No patients had taken any anti-inflammatory drugs other than aspirin for at least 10 days prior to inclusion. All patients gave their written, informed consent, and the study was approved by our institutional Review Committee.

**Study protocol.** This was a double-blinded, randomized, placebo-controlled trial. All patients received aspirin 100 mg/day for at least 10 days prior to inclusion. Preliminary studies that we performed in other patients showed that this aspirin dosage completely abolished arachidonic acid–induced platelet aggregation (unpublished observations). Patients were randomly assigned to a single 10-mg oral dose of either S 18886 (n = 12) or placebo (n = 8). S 18886 and placebo caps were provided by I.R.I.S. (Courbevoie, France). Vascular tone and endothelium-dependent vasodilation were evaluated in the forearm vascular bed before and 60 min after the S 18886 or placebo dose. Flow-mediated vasodilation of the brachial artery was measured during a hyperemia test using a high-resolution ultrasound echo-tracking system to record changes in brachial artery diameter (15). Then a catheter was inserted into the brachial artery, and FBF was recorded using VOP under baseline conditions and during subsequent brachial artery infusions of ACh, sodium nitroprusside (SNP), and norepinephrine.

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 to 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 (Db), for basal diameter). The FMD (%) was calculated as: 100 × (Dmax − Db)/Db.

**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,
The type 1 error rate was set at 5%. Between baseline and treatment values in each treatment arm, with a Simplate device (Organon Technika, Eppelheim, Germany) (16). Physical examinations, cardiovascular parameters (supine blood pressure, heart rate, and electrocardiography) were performed according to the Ivy Nelson method, on the forearm at selected baseline and 2 h after treatment with either S 18886 or placebo. 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 measurements did not differ between the S 18886 and placebo groups, respectively (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 ± 138.1 and 558.2 ± 148.5 with ACh and 3.78 ± 1.16 and 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)

France, as a continuous infusion at rates of 20, 40, and 80 μg/min; 2) SNP (Nitrate, 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 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, hematologic, 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).

Table 1. Patient Characteristics

<table>
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<th>Parameter</th>
<th>S 18886 Group</th>
<th>Placebo Group</th>
<th>p Value</th>
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<tr>
<td>(n = 12)</td>
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<tr>
<td>Age (yrs)</td>
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<td>Body mass index (kg/m²)</td>
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<td>Heart rate (beats/min)</td>
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<td>Diastolic blood pressure (mm Hg)</td>
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<td>Total cholesterol (mmol/l)</td>
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<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>
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<td>1.0 ± 0.3</td>
<td>NS</td>
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<td>Blood glucose (mmol/l)</td>
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*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.

Figure 1. The flow-mediated dilation (FMD) variations in response to hyperemia. The FMD values (expressed as the percentage of increase in brachial artery diameter following hyperemia) are shown before and after treatment with placebo or S 18886. p = 0.01 for comparisons of pre-treatment to post-treatment values in the S 18886 group.

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).
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 (29) 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.

Figure 4. The forearm blood flow (FBF) variations in response to brachial artery norepinephrine (NE) infusion. Norepinephrine (100, 500, and 1,000 pmol/min) was infused into the brachial artery, and FBF variations (expressed in ml/min per 100 ml) were recorded using venous occlusion plethysmography. The FBF variations are shown before and after S 18886 (top) or placebo (bottom). Statistical analysis was performed on the area under the curve of FBF. No significant differences were observed between the post-treatment values recorded in the S 18886 and placebo groups (analysis of covariance).
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