Aspirin Improves Endothelial Dysfunction in Atherosclerosis

Syed Husain, MBBS; Neil P. Andrews, BMBS; David Mulcahy, MD; Julio A. Panza, MD; Arshed A. Quyyumi, MD

Background—The beneficial effects of aspirin in atherosclerosis are generally attributed to its antiplatelet activities, but its influence on endothelial function remains uncertain. We hypothesized that a cyclooxygenase–dependent constricting factor contributes to the endothelial dysfunction in atherosclerosis and that its action can be reversed by aspirin.

Methods and Results—In 14 patients with coronary atherosclerosis and 5 with risk factors, we tested femoral vascular endothelial function with acetylcholine and substance P and endothelium-independent function with sodium nitroprusside before and after intravenous aspirin. Drugs were infused into the femoral artery, and Doppler flow velocity was measured. Acetylcholine-induced but not substance P–or sodium nitroprusside–induced vasodilation was lower in patients with atherosclerosis than in those with only risk factors. Aspirin had no baseline effect but improved acetylcholine-mediated vasodilation only in patients with atherosclerosis; at the peak dose, acetylcholine–mediated femoral vascular resistance index was 19% ± 5%, P = .002 lower. There was a correlation between the baseline response to acetylcholine and the magnitude of improvement with aspirin (r = .5, P = .05). Thus, patients with a depressed response to acetylcholine had greater improvement with aspirin, and vice versa. The presence of atherosclerosis was an independent determinant of improvement with aspirin. Aspirin had no effect on the responses to either substance P or sodium nitroprusside.

Conclusions—Cyclooxygenase–dependent, endothelium–derived vasoconstrictor release modulates peripheral vasodilation in patients with atherosclerosis. Improvement of endothelial dysfunction with aspirin may improve vasodilation, reduce thrombosis, and inhibit progression of atherosclerosis and provides a pathophysiological basis for the beneficial effects of aspirin in atherosclerosis. (Circulation. 1998;97:716–720.)

Key Words: endothelium–derived factors ■ aspirin ■ atherosclerosis

The beneficial effects of aspirin in reducing acute coronary and cerebrovascular events such as unstable angina, myocardial infarction, sudden cardiac death, and stroke have been attributed largely to its antiplatelet action and to its effects on thromboxane.1–3 Whether aspirin has a more profound action, particularly on endothelial function, which is pivotal in modulating tone, thrombotic potential, and atherosclerotic tendency of the blood vessel wall, remains a subject of speculation. The endothelium modulates vascular smooth muscle tone by paracrine release of dilating and constricting compounds that are secreted in response to a variety of physiological and pharmacological stimuli.4–7 Dilating factors include nitric oxide or a compound closely related to it,5 endothelium–derived hyperpolarizing factor,6 and vasodilator prostaglandins, such as prostacyclin.8 Constricting agents released by the endothelium include endothelin,9 constricting cyclooxygenase products such as thromboxane A2, prostaglandins F2α, and superoxide anion.11–17 Acetylcholine, a commonly used probe for testing endothelial function in humans, causes endothelium–dependent smooth muscle vasodilation, which is believed to be caused largely by release of nitric oxide and endothelium–derived hyperpolarizing factor.4–7 Acetylcholine–induced vasodilation is diminished in the conductance and resistance vessels of patients with atherosclerosis and in those with risk factors for atherosclerosis, such as hypercholesterolemia, hypertension, diabetes, aging, and smoking, and in patients with congestive heart failure.18–25 This abnormality is usually attributed to decreased activity of endothelium–derived relaxing factors, but recent studies show that an abnormal response to acetylcholine in congestive heart failure and diabetes may be due to production of a cyclooxygenase–dependent vasoconstrictor factor.26–30

In this study, we tested the hypothesis that the beneficial effects of aspirin in patients with atherosclerosis may be due, at least in part, to improvement of endothelial dysfunction. For this purpose, we investigated whether an endothelium–derived cyclooxygenase–dependent constricting factor contributes to the abnormal acetylcholine–mediated dilation in patients with atherosclerosis.

Methods

Patients

The study was performed at least 2 hours after patients underwent diagnostic cardiac catheterization for diagnosis of suspected or confirmed coronary artery disease. Femoral vascular endothelial function was studied in 19 patients who had angiographic evidence of coronary atherosclerosis (n = 14) or had one or more risk factors for atheroscle-
TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Atherosclerosis</th>
<th>Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>14</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.3±1.8</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>12 (92)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>229±14</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>135±18</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>37±2.2</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>5 (39)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>4 (31)</td>
</tr>
<tr>
<td>Previous smoking, n (%)</td>
<td>11 (85)</td>
</tr>
<tr>
<td>Family history of coronary artery disease, n (%)</td>
<td>6 (46)</td>
</tr>
<tr>
<td>Total No. of risk factors</td>
<td>3.1±0.4</td>
</tr>
</tbody>
</table>

Atherosclerosis (n=5). The risk factors were defined as presence of hypertension (arterial blood pressure >140/90), diabetes mellitus, history of past or continued smoking, cholesterol level >240 mg/dL, and age >60 years (Table 1). None had significant left ventricular dysfunction (ejection fraction by radionuclide ventriculography ≤40%) or evidence of heart failure. All cardiac medications were withdrawn at least 48 hours before the study; and aspirin and other cyclooxygenase inhibitors were discontinued for at least 7 days before the study.

Another group of 10 patients with coronary atherosclerosis was studied to test the reproducibility of administration of the vasodilators on two successive occasions in the femoral circulation. Informed consent was obtained from all subjects, and the protocol was approved by the National Heart, Lung, and Blood Institute Ethical Review Board.

Measurement of Femoral Blood Flow Velocity

A 6F angiographic multipurpose A2 (Cordis, Inc) catheter was introduced 1 cm beyond the end of a 7F femoral artery sheath. A 0.018-in Doppler flow wire (Cardiometrics, Inc) was introduced through the catheter 1 cm beyond the catheter tip to obtain an adequate flow velocity signal throughout the study. Drugs were infused through the sheath ≤2 cm below the tip of the Doppler wire. A femoral angiogram was performed to exclude obstructive disease in the femoral circulation. The average peak velocity during each intervention was recorded.31 Because diameter measurements were not made at the level of the Doppler wire with each intervention, we calculated the femoral vascular resistance index (FVRI, mm Hg · cm⁻¹ · s⁻¹) as the mean arterial pressure divided by femoral blood flow velocity. To exclude any significant changes in femoral artery diameter at the site of the flow wire during conditions of increased blood flow, in a preliminary study using serial angiography, we measured femoral artery diameter at the site of the flow wire during administration of 300 µg/min acetylcholine and 40 µg/min sodium nitroprusside in 24 patients. There was no significant alteration in femoral arterial diameter at the site of the flow wire during these drug infusions: baseline, 5.1±0.9 mm; acetylcholine, 5.1±0.9 mm; and sodium nitroprusside, 5.1±0.9 mm (all P=NS compared with baseline). We have also measured femoral diameter using ultrasound in the midsection of the femoral artery in a preliminary study. No constriction was observed in femoral diameter with acetylcholine in patients with atherosclerosis or its risk factors.

Study Protocol

After baseline measurement of flow velocity and mean arterial pressure, 2-minute infusions of acetylcholine at 150 and 300 µg/min were given. This was followed after a 10-minute period by administration of substance P at 20 and 40 pmol/min for 3 minutes each. The order of acetylcholine and substance P was randomized. Ten minutes after recovery and return to baseline values, sodium nitroprusside was administered at 40 µg/min for 3 to 4 minutes.

After a 15-minute recovery period and return to baseline, 1 g aspirin lysine (Aspegic injectable, Synthelabo Groupe) was infused intravenously over a 10-minute period in the study patients, and dextrose 5% was given to control patients. This was followed 15 minutes later by repeat infusions of acetylcholine, substance P, and sodium nitroprusside. Peak flow velocity and blood pressure measurements were made after each intervention.

Statistical Analysis

Data are expressed as mean±SEM. Means were compared by paired or unpaired Student’s t test, as appropriate, and discrete data were compared by the χ² test. All probability values are two-tailed. The global effects of aspirin on two doses of acetylcholine and substance P were compared by ANOVA for repeated measures.32 Multiple stepwise regression analysis33 was performed to test whether the magnitude of change in the response to the peak dose of acetylcholine with aspirin was related to age, sex, presence of hypertension, diabetes, cigarette use, cholesterol level, HDL level, family history of coronary artery disease, or the presence or absence of angiographic atherosclerosis.

Results

Vascular Responses in Patients With and Without Atherosclerosis

Acetylcholine, substance P, and sodium nitroprusside produced progressive microvascular dilation both in patients with atherosclerosis and in those with risk factors for atherosclerosis. However, the response to acetylcholine but not to substance P or to sodium nitroprusside was significantly blunted in patients with atherosclerosis (Fig 1). Thus, at the peak dose of acetylcholine, FVRI was 58±4% lower than baseline in those with risk factors and 46±5% (P=.04) lower in those with atherosclerosis. There was no change in arterial blood pressure during the infusions.

Effect of Aspirin

Fifteen minutes after intravenous aspirin, there was no significant change in systemic arterial blood pressure or FVRI in either group of patients. Aspirin significantly enhanced the vasodilation in response to acetylcholine in patients with atherosclerosis, but there was no change in those without atherosclerosis (Figs 2 and 3). At the highest dose of acetylcholine, FVRI was 19% lower in patients with atherosclerosis (P=.002), but the 1% change in those with risk factors only was not significant (Figs 2 and 3).

When the response to aspirin of all 19 patients was examined, there was a significant correlation between the percent change in FVRI with acetylcholine (300-µg/min dose) and
the magnitude of improvement in FVRI with aspirin, $R=.50$, $P=.05$, indicating that patients with an initially lower response to acetylcholine had greater improvement with aspirin than those with a higher vasodilator response to acetylcholine. The latter had no significant change with aspirin.

Multivariate analysis was performed in all 19 patients to examine whether the presence of angiographic atherosclerosis or any risk factor for atherosclerosis (age, sex, serum cholesterol level, HDL level, ejection fraction, presence of hypertension, diabetes, family history of coronary artery disease, or smoking history) determined the magnitude of the effect of aspirin on the acetylcholine response. The only significant predictor of improvement in the FVRI with acetylcholine after aspirin was the presence of angiographic atherosclerosis, $r=.60$, $P=.005$.

In contrast to its effect on the acetylcholine response in patients with atherosclerosis, aspirin produced no change in the vasodilator responses to either substance P or sodium nitroprusside in either patients with or those without atherosclerosis (Figs 2 and 3). At the peak dose of substance P, aspirin produced a 4.5%, $P=.26$ decrease in FVRI in patients with atherosclerosis and a 16%, $P=.12$ increase in those with risk factors. Similarly, with sodium nitroprusside, aspirin produced a 1%, $P=.95$ reduction in FVRI in those with atherosclerosis and a 6%, $P<.53$ reduction in those without atherosclerosis (Figs 2 and 3).

Reproducibility of Vasodilator Infusions
To examine whether two successive infusions of acetylcholine and substance P produced similar vasodilation in the femoral circulation, we tested responses to them in 10 patients before and after dextrose 5% (Table 2). The effect of the first infusion of both endothelium-dependent vasodilators was reproducible during the second infusion after dextrose 5%.

### Table 2. Reproducibility of Changes in Femoral Vascular Resistance Index With Acetylcholine and Substance P in Control Patients

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dextrose 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Rest</td>
<td>Dextrose 5%</td>
</tr>
<tr>
<td>Acetylcholine 150 µg/min</td>
<td>4.4±7.2</td>
<td>5.3±2.5</td>
</tr>
<tr>
<td>Acetylcholine 300 µg/min</td>
<td>3.9±1.7</td>
<td>4.1±1.7</td>
</tr>
<tr>
<td>Rest</td>
<td>6.5±2.3</td>
<td>6.8±2.6</td>
</tr>
<tr>
<td>Substance P 10 pmol/min</td>
<td>3.3±0.9</td>
<td>3.5±0.9</td>
</tr>
<tr>
<td>Substance P 20 pmol/min</td>
<td>2.9±0.8</td>
<td>3.3±0.9</td>
</tr>
</tbody>
</table>

Changes between control and dextrose 5% were not significant by ANOVA.

### Discussion
The major finding of this study is that aspirin modulates acetylcholine-induced peripheral vasodilation in patients with atherosclerosis, possibly via inhibition of one or more cyclooxygenase–dependent vasoconstrictors. There is no evidence for significant production of either vasodilator or vasoconstrictor prostaglandins in response to the nonmuscarinic endothelium-dependent vasodilator substance P or with the endothelium-independent dilator sodium nitroprusside.

Release of vasoconstrictor prostaglandins by acetylcholine, an endothelium–dependent agonist that also releases nitric oxide and other vasodilators, has been demonstrated in animal studies.11–17 Endothelial impairment in rat aortic rings was accompanied by increased production of thromboxane A$_2$ and prostaglandin F$_{2α}$, an abnormality that was normalized by inhibitors of cyclooxygenase or prostaglandin endoperoxide.12–14,27 Acetylcholine-induced, endothelium–dependent contractions of aortic strips from spontaneously hypertensive rats and pulmonary arterioles of rabbits were inhibited by indomethacin.13,14 Release of acetylcholine-mediated, cyclooxygenase–dependent, endothelium–derived vasoconstrictor substances was observed in the canine model of heart failure,30 a finding that was subsequently corroborated in the peripheral circulation of patients with congestive heart failure. Cyclooxygenase inhibition with indomethacin increased acetylcholine–mediated forearm vasodilation by 39% in patients with congestive heart failure but not in normal subjects.26,29 Despite demonstration of acetylcholine–mediated release of vasoconstrictor prostaglandins in animal models of hypertension and diabetes,13,14,27,28,34 most human studies have failed to show any effect of cyclooxygenase inhibitors on acetylcholine–mediated forearm vasodilation in hypertensive and diabetic patients,26,36 although one study recently demonstrated release of constrictor prostaglandins with acetylcholine in the forearm circulation of patients with hypertension.37 In agreement with these reports, patients with risk factors but without atherosclerosis in our study group also had no apparent effect with aspirin, and none of our patients had evidence of congestive heart failure. To the best of our knowledge, our study is the first demonstration of acetylcholine–mediated constrictor prostaglandin production in response to acetylcholine in patients with atherosclerosis.

Multivariate analysis failed to identify a single risk factor or a combination of risk factors that were associated with the

### Figure 2. Effect of aspirin on response to acetylcholine (left), substance P (middle), and sodium nitroprusside (right) in 14 patients with atherosclerosis. Abbreviation as in Fig 1.

### Figure 3. Effect of aspirin on response to acetylcholine (left), substance P (middle), and sodium nitroprusside (right) in 5 patients with risk factors but without angiographic atherosclerosis. Abbreviation as in Fig 1.
magnitude of improvement of the acetylcholine response with aspirin. However, the presence of atherosclerosis was an independent predictor of improvement with cyclooxygenase inhibition. Patients with atherosclerosis also had a lower vasodilator response with acetylcholine compared with patients without risk factors but without atherosclerosis in this study and also compared with patients without risk factors studied in a previous investigation.41 Taken together, these findings suggest that patients with worse endothelial function, as indicated by a greater depression of the acetylcholine response, had greater production of constrictor prostaglandins. Thus, as atherosclerosis develops in patients with risk factors and endothelial dysfunction progresses, there appears to be significant production of constrictor substances in response to muscarinic receptor stimulation.

Our study also demonstrated that the response to the nonmuscarinic endothelium-dependent vasodilator substance P in patients with atherosclerosis did not correlate with the response to acetylcholine, a finding observed previously in the coronary vasculature.28,39 Because the second messenger pathways from the activation of tachykinin and muscarinic receptors are believed to be the same,40 it is possible that the differences observed are secondary to differential dysfunction of the endothelial surface receptors by factors that produce atherosclerosis. There was little evidence for either constrictor or dilator prostaglandin production in response to substance P, unless both were generated in quantities that resulted in no net vascular effect. Thus, the depression in the acetylcholine response observed in patients with intact substance P–mediated vasodilation may also be due to stimulation of vasoconstrictor prostanoids by acetylcholine, in addition to factors mentioned above.

We did not attempt to characterize the nature of the vasoconstrictor released in response to acetylcholine in patients with atherosclerosis in this study. Possible mediators include prostaglandin F, thromboxane A, and related vasoconstricators prostaglandins.41–17 A more provocative and recently elucidated antioxidant action of cyclooxygenase inhibitors may be an important mechanism for the observed effect of aspirin in this study. Salicylic acid is known to scavenge both oxygen free radicals and hydroxyl radicals in activated granulocytes,41 and acetylcholine-mediated constriction due to cyclooxygenase-dependent superoxide anion production has been demonstrated in several animal models.42–45 Superoxide anions avidly metabolize nitric oxide to higher, biologically inactive nitrogen oxides and are believed to be the primary mechanism underlying the reduced bioavailability of nitric oxide in atherosclerosis. Thus, it is possible that as endothelial dysfunction progresses in atherosclerosis, acetylcholine but not substance P promotes increased production of cyclooxygenase-dependent free radicals, which in turn inactivate nitric oxide and reduce its bioavailability.

Limitations
The relatively small group of patients without atherosclerosis but with risk factors included in this study may have obscured a potential effect of aspirin in this population. Also, there may be other risk factors that produce endothelial dysfunction, such as homocysteinuria, that were not measured in these patients. However, our findings are compatible with the lack of effect of cyclooxygenase inhibitors on the acetylcholine response in patients with hypertension or diabetes.33,36 Furthermore, the inverse correlation between baseline vasodilation with acetylcholine and the magnitude of improvement with aspirin suggests that if the acetylcholine response in subjects with risk factors reduces to the level observed in patients with atherosclerosis, then aspirin is also likely to produce improvement.

Although we have assumed that the beneficial effects of aspirin are most likely due to its effects as a cyclooxygenase inhibitor, it is possible that there may be as yet unidentified mechanisms that may produce the observed changes.

Implications
The clinical implications of our findings are likely to be important for patients with atherosclerosis, because there is a relationship between the response to pharmacological stimulation of the vascular endothelium with acetylcholine and the physiological vasodilation in response to metabolic stresses such as atrial pacing,46,47 mental stress,48 exercise,49 and hyperemia50; thus, patients with an abnormal response to acetylcholine also tend to have abnormal vasodilation in response to these physiological stimuli. Whether there is a contribution from endothelium-dependent constrictor factor release to the abnormal flow-mediated vasodilation observed in atherosclerotic blood vessels, which has hitherto been believed to be largely due to reduced nitric oxide activity, needs further investigation. Moreover, it is conceivable that aspirin may also improve physiological vasodilation in this population, a hypothesis that requires further evaluation. Finally, if the effect of aspirin on improving endothelial dysfunction is mediated by reduction in oxidant stress by inhibition of cyclooxygenase–dependent free radical production in the vessel wall, it is likely that over the long term, increased nitric oxide bioavailability in atherosclerotic blood vessels will reduce their thrombogenic tendency and decelerate progression of plaque development.51,52 Thus, demonstration of improved endothelium–dependent vasodilation with aspirin provides a novel pathophysiological basis for its beneficial effects in atherosclerotic patients, which to date has been largely attributed to its antithrombotic effects.

References


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