Short- and Long-Term COX-2 Inhibition Reverses Endothelial Dysfunction in Patients With Hypertension


Abstract—Hypertension is associated with endothelial dysfunction that is attributable to oxidative stress and a proinflammatory state. Under these conditions, enhanced expression of cyclooxygenase-2 might lead to increased production of vasoconstrictor prostanoids and reactive oxygen species that reduce the availability of endothelium-derived nitric oxide. To investigate the contribution of cyclooxygenase-2 activity to endothelial dysfunction in human hypertension, we evaluated brachial artery vasodilator function by ultrasound in 29 hypertensive patients before and after treatment with the selective cyclooxygenase-2 inhibitor celecoxib or placebo in a randomized, double-blind study. Brachial artery flow–mediated dilation improved from a baseline of 7.9±4.5% to 9.9±5.1% (P=0.005) 3 hours after the first dose and to 10.1±6.1% (P=0.006) after 1 week of treatment with celecoxib. In contrast, placebo treatment had no significant effect on flow-mediated dilation (8.1±4.4%, 8.3±3.5%, and 8.0±3.2%, respectively). Neither treatment altered nitroglycerin-mediated dilation, extent of reactive hyperemia, or baseline arterial diameter. Celecoxib treatment had no significant effect on the urinary concentrations of F2 isoprostane or thromboxane metabolites. However, urinary concentrations of the prostacyclin metabolite 2,3-dinor-6-ketoprostaglandin F1α were significantly lower after 1 week of celecoxib treatment. Thus, cyclooxygenase-2 products contribute to endothelial dysfunction in hypertension, and treatment with a cyclooxygenase-2 inhibitor could have a beneficial effect in this setting. However, cyclooxygenase-2 inhibition also has an adverse effect on prostacyclin production that could promote thrombosis, and the net clinical consequences of improved endothelial function versus loss of prostacyclin merits further investigation. (Hypertension. 2003;42:310-315.)

Key Words: cyclooxygenase ■ endothelium ■ prostacyclin ■ hypertension, essential

The vascular endothelium maintains normal vascular homeostasis by producing paracrine factors that regulate vascular tone, thrombosis, intimal growth, and entry of inflammatory cells into the vascular wall.1 Hypertension is associated with an altered endothelial phenotype in resistance vessels and conduit arteries that might contribute to blood pressure elevation and to the development of atherosclerosis.1-3 Risk of cardiovascular disease events in hypertensive patients is independently associated with endothelial dysfunction in forearm resistance vessels.4 Furthermore, cardiovascular risk is reduced when endothelium-dependent, flow-mediated dilation of the brachial artery improves after initiation of antihypertensive therapy.5

There is great interest in defining the causes of endothelial dysfunction in hypertension. Experimental studies suggest that production of vasoconstrictor substances by cyclooxygenase (COX), including vasoconstrictor prostanoids and reactive oxygen species, contribute to the pathogenesis of endothelial dysfunction in this disease.6,7 Reactive oxygen species generated by COX reduce the biologic activity of endothelium-derived nitric oxide (NO) directly and indirectly by contributing to lipid peroxidation, products of which might also decrease NO synthesis and bioavailability.8,9 In support of these mechanisms, studies in human hypertension have demonstrated improved endothelium-dependent dilation after treatment with nonselective COX inhibitors, including aspirin and indomethacin,10-12 and with antioxidants capable of scavenging reactive oxygen species.8,13

There is growing recognition of the importance of inflammation in the pathogenesis of cardiovascular disease,14,15 and one component of the inflammatory response is increased expression of COX-2.16 Under conditions of inflammation and increased oxidative stress, increased generation of reactive oxygen species and formation of peroxynitrite further activate COX-2 and inhibit the activity of prostacyclin synthase.17-19 The result might be increased and preferential production of vasoconstrictor prostanoids and impaired NO bioactivity in the vascular wall. We therefore hypothesized

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that COX-2 plays a particularly important role in the endothelial dysfunction associated with hypertension and that treatment with a selective COX-2 inhibitor would improve endothelial function and reduce oxidative stress in hypertensive patients.

**Methods**

**Patient Population**

We recruited otherwise healthy hypertensive volunteers by advertisement. Hypertension was defined clinically as ongoing antihypertensive therapy prescribed by the subject’s physician or blood pressure ≥140/90 measured on 3 occasions. Patients with clinically defined coronary artery disease, diabetes mellitus (fasting glucose >125 mg/dL), or hypercholesterolemia (LDL cholesterol >160 mg/dL or on therapy) were excluded. No volunteer took aspirin or nonsteroidal anti-inflammatory drugs within 2 weeks of entering the study. We excluded volunteers taking antioxidant vitamins <1 month before entering the study. All volunteers gave written consent, as approved by the Boston Medical Center Institutional Review Board.

**Study Protocol**

Each enrolled volunteer withheld all vasoactive medications for 48 hours, stopped smoking for 12 hours (if applicable), and fasted for 12 hours before each study. Heart rate, systolic blood pressure, and diastolic blood pressure were measured, and urine and venous blood samples were obtained for biochemical analysis. Endothelium-dependent, brachial artery flow-mediated dilation and endothelium-independent, nitroglycerin-mediated dilation were assessed by ultrasound. After baseline measurements, computer-generated random numbers were used to assign volunteers to either celecoxib 200 mg or placebo 2 times per day for 1 week in a double-blind fashion. We repeated measurement of vital signs and vascular function 3 hours after the first dose, at the time of peak serum levels.16 We also collected repeated blood and urine samples and remeasured vascular function after 1 week of treatment to gain insight into the more long-term effects of therapy. The last dose of study medication was administered the evening before the follow-up visit.

**Assessment of Vascular Function**

Flow-mediated and nitroglycerin-mediated dilation (0.4 mg sublingual) and hyperemic flow of the conduit brachial artery were determined by use of high-resolution vascular ultrasound and an upper-arm occlusive cuff as previously described.20,21 Nitroglycerin was administered by use of high-resolution vascular ultrasound and an upper-arm occlusive cuff as previously described.20,21 Nitroglycerin was omitted when systolic blood pressure was <100 mm Hg or the volunteer reported previous adverse reaction to nitroglycerin, history of migraines, or sildenafil citrate use within 1 week of study. Ultrasound images were digitized and stored. Blinded analysis was performed with commercially available software (Brachial Analyzer, Medical Imaging Applications).

**Biochemical Analysis**

Serum total cholesterol, HDL cholesterol, triglycerides, and glucose and urine creatinine were measured by automated analyzer in the Boston Medical Center Clinical Laboratory (Hitachi-917). LDL cholesterol was calculated with the Friedewald formula. We used gas chromatography–mass spectroscopy to measure the F2 isoprostane metabolite 2,3-dinor-5,6-dihydro-8-iso-prostaglandin F2α (IsoP-M), the prostacyclin metabolite 2,3-dinor-6-keto-PDF2α, and the thromboxane B2 metabolite 11-dehydrothromboxane B2 (11-dehydro-TxB2) in “spot” urine samples that had been stored for up to 18 months at −80°C.22–24 Urine content of these products is expressed as nanograms per milligram creatinine.

**Statistical Analysis**

Data are presented as mean±SD except in the figures, where the data are presented as mean±SEM. All statistical analyses were performed with SPSS 10.1 software (SPSS Inc). Baseline characteristics were compared with a Mann-Whitney U test, χ2, or Fisher exact tests, as appropriate. The effects of treatment on flow-mediated dilation, nitroglycerin-mediated dilation, lipid peroxidation, and levels of PG breakdown products were compared by 2-way repeated-measures ANOVA, with the Greenhouse-Geisser correction as needed for nonspherical data. Post hoc multiple pairwise comparisons were performed with the Student-Newman-Keuls procedure when the ANOVA time-treatment interaction was significant. Power analysis indicated that a sample size of 15 patients per group (celecoxib and placebo) provided 80% power to detect a change of 0.1 mm and of 2.0 percentage points for brachial artery diameter and flow-mediated dilation, respectively (α=0.05).

**Results**

**Clinical Characteristics**

We enrolled 38 subjects who met the eligibility criteria. Two subjects withdrew, and 7 had 1 or more ultrasound studies that were technically unsuitable, leaving 29 patients available for analysis. Baseline characteristics are presented in Table 1. There were no significant differences between the 2 groups with regard to any of these characteristics, although there was a trend toward a lower mean age in the celecoxib group (P=0.06).

**Effect of Treatment on Vascular Function**

Table 2 outlines vital signs and brachial artery parameters for the 3 timepoints. At baseline, heart rate, blood pressure, resting brachial artery diameter, and extent of reactive hyperemia were equivalent between groups. There were no changes over time and no significant treatment-time interactions for these parameters.

Figure 1 summarizes the flow-mediated dilation results. Flow-mediated dilation was equivalent at baseline in the celecoxib and placebo groups (7.9±4.5% and 8.1±4.4%, respectively; P=0.48 at baseline). However, flow-mediated dilation significantly increased 3 hours after treatment with celecoxib, to 9.9±5.1% (P=0.005), and to 10.1±6.1% (P=0.006) after 1 week of treatment in the celecoxib group. Compared with baseline, flow-mediated dilation was unchanged after short- (8.3±3.5%; P=0.82) and long-term (8.0±3.2%; P=0.80) term placebo treatment. Overall, the
response to celecoxib differed from the response to placebo by repeated-measures ANOVA (time-treatment interaction \( P = 0.04 \)).

Figure 2 displays the nitroglycerin-mediated dilation results. Nitroglycerin responses were equivalent at baseline in the celecoxib and placebo groups (17.6\( \pm \)6.1\% and 18.7\( \pm \)7.1\%). Nitroglycerin-mediated dilation was 18.9\( \pm \)6.2\% and 18.8\( \pm \)7.4\% in the placebo group and 15.6\( \pm \)5.4\% and 17.3\( \pm \)5.9\% in the celecoxib group after short- and longer-term treatment, respectively. Overall, the nitroglycerin-mediated dilation responses over time did not differ between treatment groups (time-treatment interaction \( P = 0.46 \)).

**Effect of Treatment on Urinary Prostanoids**

Stable urinary metabolites of prostacyclin (2,3-dinor 6-keto-PGF\(_{1\alpha}\)), thromboxane A\(_2\) (11-dehydro-TxB\(_2\)) and F\(_2\) isoprostanes (IsoP-M) were measured in a subset of the study population at baseline and after 1 week of treatment. 11-dehydro-TxB\(_2\) (n = 6 for celecoxib, n = 9 for placebo) and IsoP-M (n = 6 for celecoxib, n = 9 placebo) did not change over time in the 2 treatment groups (time-treatment interaction \( P = 0.62 \) and \( P = 0.46 \), respectively). 11-dehydro-TxB\(_2\) levels before and after placebo and before and after celecoxib therapy were 0.20\( \pm \)0.15, 0.21\( \pm \)0.16, 0.41\( \pm \)0.15, and 0.37\( \pm \)0.34 \( \text{ng/mg creatinine} \), respectively. IsoP-M levels before and after placebo and before and after celecoxib therapy were 1.18\( \pm \)0.61, 1.33\( \pm \)0.98, 1.01\( \pm \)0.43, and 0.88\( \pm \)0.34 \( \text{ng/mg creatinine} \), respectively. However, as shown in Figure 3, there was a significant effect of treatment on 2,3-dinor-6-keto-PGF\(_{1\alpha}\) levels (n = 7 for celecoxib, n = 10 for placebo; time-treatment interaction \( P = 0.03 \)). This effect was attributable to a post-celecoxib treatment decrease in the 2,3-dinor-6-keto-PGF\(_{1\alpha}\) levels, from 0.36\( \pm \)0.16 to 0.18\( \pm \)0.09 \( \text{ng/mg creatinine} \) in the celecoxib group (\( P = 0.004 \)), whereas the levels were similar before and after placebo treatment (0.30\( \pm \)0.19 and 0.34\( \pm \)0.22, respectively).

**Discussion**

This study demonstrated a significant improvement in brachial artery flow–mediated dilation after treatment with the selective COX-2 inhibitor celecoxib in hypertensive patients with neither diabetes nor hypercholesterolemia. There were no effects of treatment on nitroglycerin-mediated dilation, brachial artery diameter, or reactive hyperemia, suggesting that celecoxib selectively improved endothelial function and did not alter the general vasodilator capacity of the brachial artery or the stimulus for dilation. At baseline, flow-mediated dilation was comparable to that observed in prior studies of hypertensive patients\(^{25}\) but lower than that observed in healthy subjects.\(^{25,26}\) These findings suggest that COX-2

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**TABLE 2. Brachial Artery, Hemodynamic, and Biochemical Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Celecoxib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3 Hours</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>68( \pm )8</td>
<td>66( \pm )11</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>142( \pm )10</td>
<td>141( \pm )11</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>84( \pm )10</td>
<td>85( \pm )9</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>4.48( \pm )0.70</td>
<td>4.46( \pm )0.70</td>
</tr>
<tr>
<td>Hyperemic diameter, mm</td>
<td>4.83( \pm )0.69</td>
<td>4.82( \pm )0.66</td>
</tr>
<tr>
<td>Hyperemic flow, % increase</td>
<td>514( \pm )340</td>
<td>571( \pm )330</td>
</tr>
</tbody>
</table>

Data are mean\( \pm \)SD. No significant differences were found. HR indicates heart rate; DBP, diastolic blood pressure; and SBP, systolic blood pressure.
products contribute to the pathogenesis of endothelial dysfunction in the conduit vessels of patients with hypertension.

We observed no effect of treatment on systemic thromboxane production and a significant decrease in prostacyclin production after 1 week of celecoxib treatment. The former finding argues against an important role for enhanced production of the vasoconstrictor thromboxane as a mechanism of impaired flow-mediated dilation in hypertension, although we cannot rule out that a modest reduction in thromboxane production could have occurred with COX-2 inhibition, given the small sample size. The reduction in prostacyclin production is consistent with prior studies and confirms that celecoxib had a biologic effect. However, this result suggests that prostacyclin-mediated vasodilation does not contribute substantially to conduit-artery flow-mediated dilation in hypertensive patients. Finally, urinary levels of \( \text{F}_{2} \text{Isoprostanes (IsoP-M)} \) were not materially altered by COX-2 inhibition, suggesting that inhibition of systemic lipid peroxidation is not a major mechanism for the effect of celecoxib. These findings are consistent with a recent report that hypertension is not an important source of systemic lipid peroxidation in the Framingham Heart Study. However, a modest reduction in oxidative stress by COX-2 inhibition cannot be excluded by this study, given our small sample size. Furthermore, it is possible that examination of alternative markers of systemic oxidative stress might have provided evidence to support this hypothesis.

It should also be noted that these systemic markers of prostaglandin synthesis and oxidative stress might not reflect the operative mechanisms in the vascular wall. It remains possible that treatment with a COX-2 inhibitor reduces local production of superoxide anion and reactive nitrogen species and increases the bioavailability of endothelium-derived NO. It also seems likely that COX-2 inhibition leads to a reduction in the local production of vasoconstrictor and/or proinflammatory prostanooids that contribute to impaired vasodilator function in hypertension. Further study of these mechanisms was beyond the scope of the present human study.

No previous study has examined the effects of a COX-2 inhibitor on vascular function in hypertensive patients. However, a prior study examined this question in the conduit brachial artery of patients with coronary artery disease. Consistent with the present study, Chenevard and colleagues reported an improvement in brachial artery flow-mediated dilation after 2 weeks of celecoxib treatment. Interestingly, treatment in that study was also associated with reductions in C-reactive protein and plasma levels of oxidized LDL. This latter finding conflicts with our observation that urinary IsoP-M levels were not affected by celecoxib treatment, but this discrepancy might be attributable to differences in the specific marker of lipid peroxidation, as well as differences in the patient populations (patients with coronary disease versus mild hypertension). Interpretation of the study by Chenevard and colleagues was complicated by the ongoing aspirin treatment in all subjects, which was unavoidable, given the presence of coronary artery disease. For example, nonspecific COX inhibition by aspirin might have reduced baseline levels of prostacyclin and thromboxane and account for their observation that celecoxib treatment had no further effect on systemic prostacyclin. The present investigation of patients with hypertension provided an opportunity to specifically examine the role of COX-2 in vascular dysfunction.

Our findings are consistent with several prior studies that examined the effects of nonselective COX inhibitors in resistance vessels of patients with hypertension and atherosclerosis. For example, Taddei and colleagues observed that indomethacin improved acetylcholine-mediated dilation of resistance vessels of patients with hypertension and provided evidence that this improvement was attributable to increased bioavailability of endothelium-derived NO. Similarly, Campia and colleagues observed improved endothelium-dependent vasodilation in forearm resistance vessels of hypertensive patients after aspirin treatment, and this response likely reflected reversal of endothelial dysfunction.

Husain and colleagues observed improved endothelial vasodilator function in lower-extremity resistance vessels of patients with atherosclerosis after intravenous aspirin treatment.

It might be argued that our findings could reflect an alteration of normal physiologic control of vascular tone and that celecoxib might also improve endothelium-dependent vasodilation in healthy subjects. Because we did not study healthy subjects, we cannot exclude this possibility. However, Verma and colleagues reported that long-term oral administration of the selective COX-2 inhibitor rofecoxib had no effect on endothelium-dependent or endothelium-independent vasodilation in the forearm circulation of healthy subjects with normal blood pressure and no coronary risk factors. Although that study examined resistance vessels rather than conduit vessels, the results support our contention that COX-2 products play a pathophysiologic role in vascular dysfunction in hypertension.

Our study has several other potential limitations. We enrolled otherwise healthy patients with well-controlled hypertension, and as such, the results reported here might not extend to patients with additional risk factors, such as hypercholesterolemia and diabetes mellitus. Blood pressure was only modestly elevated in our subjects, possibly reflecting the residual effects of antihypertensive therapy that had been withheld for 48 hours before study. Despite the potential...
beneficial effects of antihypertensive drugs on endothelial function, we were able to demonstrate improved function with COX-2 inhibition. There was a trend toward a younger age in the treatment group despite the randomized study design, a finding that likely reflects the relatively small sample size. However, there is no apparent reason as to how a modest group difference in age could account for our findings. Finally, our findings with regard to urinary prostanooids came from only a subset of subjects, and our negative findings with regard to F$_2$ isoprostanes and 11-dehydro-TxB$_2$ need to be verified by a larger study group.

**Perspectives**

The present study has several clinical implications. Extensive previous work has shown that impaired endothelium-dependent vasodilation in the coronary and forearm circulation is associated with increased cardiovascular disease risk (see our recent review$^{32}$). Impaired brachial artery flow-mediated dilation is specifically associated with increased short-term$^{33}$ and long-term$^{34}$ risk in peripheral arterial disease patients. Importantly, reversal of endothelial dysfunction after initiation of antihypertensive therapy is associated with reduced cardiovascular risk in patients with hypertension.$^5$ In light of this prior work, the findings of the present study suggest that COX-2 inhibition might have a beneficial effect on cardiovascular disease risk in hypertensive patients. Such an effect has been proposed as a potential explanation for the findings of the recent NUT-2 study, which suggested a beneficial effect of a COX-2 inhibitor given in combination with aspirin and heparin to patients with acute coronary syndromes.$^{35,36}$

On the other hand, our study demonstrated that celecoxib treatment was associated with a potentially disadvantageous shift in systemic prostaglandin synthesis (decreased prostacyclin with unchanged thromboxane) that might predispose patients to thrombotic events.$^{36,37}$ The beneficial effects of an intervention on endothelial function, such as COX-2 inhibition, must be viewed and studied in the context of other biologic effects of the intervention that might attenuate its benefits. Such “other biologic effects” likely explain the lack of efficacy of hormone replacement therapy in reducing cardiovascular risk in patients with hypertension.$^5$ Therefore, further randomized studies will be required to determine the net clinical effects of selective and/or nonselective COX-2 inhibition.$^{36}$

In summary, our study demonstrated that treatment of hypertensive patients with a selective COX-2 inhibitor partially reverses conduit-vessel endothelial dysfunction. These findings provide insight into the causes of vascular dysfunction in hypertension. Furthermore, our results raise the possibility that COX-2 inhibition could be beneficial for patients with hypertension, but randomized clinical trials will be required to definitively address this possibility and the relative merits of selective and nonselective COX inhibition.

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**References**


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