Effect of Low-Dose Aspirin on Thromboxane Production
and the Antihypertensive Effect of Captopril

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ABSTRACT

Some of the antihypertensive effects of angiotensin-converting enzyme (ACE) inhibitors occur through nonangiotensin II-mediated mechanisms. One of these is through decreased kinin degradation, leading to enhanced production of vasodilator arachidonic acid metabolites. It was reasoned that if ACE inhibition also leads to an increase in the production of the potent vasoconstrictor thromboxane A2, then maneuvers that selectively inhibit thromboxane production without reducing prostaglandins (PG) E2 + PGI2 might enhance the antihypertensive effect of ACE inhibition. This double-blinded, randomized, crossover study was therefore undertaken to determine: (1) if captopril increases platelet and/or renal thromboxane production; and (2) if low-dose aspirin enhances the antihypertensive effect of captopril. Patients with mild essential hypertension and no other significant medical problems were studied. In a double-blinded, random order, patients took captopril alone (25 mg every 12 h) for 2 wk and captopril plus aspirin (75 mg/day) for another 2 wk. Active treatment periods were preceded by 2 wk of single-blind placebo. Fifteen patients with a mean age of 53 yr and an average mean arterial pressure (MAP) of 114 ± 8 (±SD) mm Hg were studied. Serum thromboxane B2 was higher (P < 0.05) during treatment with captopril/placebo (600 ± 46 (±SE) pg/mL) than during the two washout periods combined (420 ± 57 and 553 ± 78) and was lowest (P < 0.0005) during treatment with captopril/aspirin (302 ± 36). Captopril treatment significantly increased the urinary excretion of PGE2 (P = 0.038). Captopril/placebo significantly lowered MAP (P < 0.05) to 105.0 ± 3.7 mm Hg compared with the washout period. However, the addition of aspirin to captopril caused no additional lowering of MAP (105.2 ± 2.8 mm Hg). It was concluded that treatment with captopril does increase platelet thromboxane production. However, lowering platelet thromboxane with low doses of aspirin may not enhance the antihypertensive effect of captopril.

Key Words: Angiotensin-converting enzyme, blood pressure, prostaglandin, eicosanoid, kinin

Angiotensin-converting enzyme inhibitors (ACEI) are effective antihypertensive drugs in many forms of hypertension. Although some of the blood pressure–lowering effects of these agents depend on the inhibition of angiotensin II production, it has become clear that additional antihypertensive effects are mediated by nonangiotensin II–dependent mechanisms (1,2). ACE and kininase II are the same enzyme, discovered and named by separate sets of investigators (2). Thus, the inhibition of ACE not only reduces angiotensin II production, but it also increases tissue concentrations of bradykinin. The resulting enhanced stimulation of B2 bradykinin receptors causes the release of arachidonic acid from cell membranes (1,2). The additional metabolism of arachidonic acid through the cyclooxygenase pathway can lead to overproduction of the vasodilator prostaglandins (PG) PGE2 and PGI2 and possibly of the vasoconstrictor thromboxane (TX)A2, as shown in Figure 1 (3–5).

In support of a role for vasodilator prostaglandins in the antihypertensive mechanism of ACEI, several investigators have shown that the pharmacologic inhibition of prostaglandin production blunts or abolishes the antihypertensive effect of ACEI in patients with low renin hypertension (5–9). Those studies have used doses of aspirin or other cyclooxygenase inhibitors that inhibit both vasodilator and vasoconstrictor eicosanoid production. If the bradykinin stimulation resulting from ACEI also increases the production of the vasoconstrictor TXA2, then the antihypertensive effect of ACEI might be enhanced by maneuvers that selectively inhibit thromboxane production without reducing the levels of the vasodilators (PGE2, PGI2).

In healthy humans, it is unclear whether the potent vasoconstrictor TXA2 is an important contributor to
the regulation of systemic vascular tone. However, some clinical studies support a role for thromboxane in the pathogenesis of hypertension. For example, the inhibition of platelet cyclooxygenase with low-dose aspirin helps to prevent pregnancy-induced hypertension in women at risk, while selectively decreasing thromboxane production (10,11). In patients with essential hypertension, fish oil supplements lower blood pressure, decrease the excretion of TXA2 metabolites, and increase the excretion of metabolites of the less potent TXA3 (12). These studies indicate that, at least in these forms of human hypertension, blood pressure may be influenced by the ratio of vasodilator to vasoconstrictor eicosanoids.

We therefore undertook a double-blind, randomized, crossover study to determine: (1) if ACEI increases platelet and/or renal thromboxane production; and (2) if low-dose aspirin enhances the antihypertensive effect of ACEI. Aspirin in a dose of 75 mg/day was chosen because it selectively inhibits platelet thromboxane production without significantly altering vasodilator prostaglandin production (13-15). Moreover, aspirin is commonly used for the prevention of thrombotic events in patient populations in which ACEI are also frequently prescribed for the treatment of hypertension and congestive heart failure (16-18). Captopril was chosen on the basis of data suggesting that the sulfhydryl group is important in the enhancement of prostaglandin formation by ACEI (1). We used the recommended starting dose for captopril in mild hypertension, 25 mg every 12 h. The crossover design was selected to maximize the power to detect a significant effect in a small number of patients.

METHODS

Patient Selection

Patients older than 18 yr of age were considered for inclusion if their seated diastolic blood pressure was 95 to 115 mm Hg on two consecutive measurements, taken at least 24 h apart with a mercury sphygmomanometer. Secondary causes of hypertension were specifically ruled out when clinically warranted according to previously published criteria (19). We also excluded patients with insulin-dependent diabetes mellitus, a serum creatinine level of more than 1.8 mg/dL, and a history of congestive heart failure, myocardial infarction, stroke, transient ischemic attack, bleeding diathesis, peptic ulcer disease, or any condition that would have prevented abstinence from aspirin and nonsteroidal anti-inflammatory medication for the duration of the study. Women with child-bearing potential were also excluded. The study was approved by the Institutional Review Board and written informed consent was obtained.

Maneuver

The study consisted of four consecutive 2-wk treatment periods, as shown schematically in Figure 2.
All antihypertensive medication was discontinued except as described below. For the first and third periods, which were single-blind washout periods, all patients received placebo capsules to be taken orally every 12 h. During the second and fourth periods, all patients instead received identical capsules containing captopril (25 mg) with the same instructions. In addition, during the second period, all patients received a second capsule with a different appearance that contained either 75 mg of aspirin or placebo as determined by a double-blind randomization scheme. These were to be taken each morning. Subsequently, during the fourth treatment period, patients were crossed over to the alternate treatment (aspirin or placebo). Thus, each subject took captopril plus placebo for 2 wk and captopril plus aspirin for 2 wk, with these two active treatment periods occurring in random order, preceded by 2 wk of single-blind placebo.

Patients remained on their usual diets and were seen at the same time of day each week, at least 3 h after taking their study medication, for the duration of the study. At each visit, blood pressure was measured in the same arm with the patient seated with a random zero sphygmomanometer (Hawksley, Lancing, United Kingdom). Mean arterial pressure (MAP) was determined by the mean of three readings taken at 5-min intervals by the use of the formula \[
\text{MAP} = \frac{\text{SBP} + (2 \times \text{DBP})}{3}
\]
(where SBP is systolic blood pressure and DBP is diastolic blood pressure). At the end of each 2-wk period, a 12-h collection of urine was performed for the measurement of eicosanoids. Blood was sampled for BUN, creatinine, potassium, and glucose via a 21-gauge steel butterfly cannula placed in the antecubital vein. The tourniquet was released and the cannula was left in place for 2 min, after which an additional sample was taken for the measurement of eicosanoids.

**Laboratory Analyses**

Eicosanoid metabolites were extracted from urine, plasma, or serum as previously described (20). Samples from urine were then subjected to separation by HPLC with a Waters Model 840 system (Millipore, Milford, MA) and a Pecosphere HS3-C 18 column (Perkin Elmer, Norwalk, CT). Eicosanoids were eluted with a linear gradient from 100% 0.017 M orthophosphoric acid to 100% acetonitrile over 10 min at 3 mL/min. Appropriate fractions were collected on the basis of retention times of known standards. The eluate was dried under nitrogen and resuspended in RIA buffer, as were the samples extracted from blood. TXB₂, PGE₂, and 6-keto-PGF₁₀ were then quantitated by RIA as previously described (20,21). Results are corrected for recovery and expressed in picograms per milligram of creatinine for urine specimens and picograms per milliliter for serum or plasma.

**Statistical Analysis**

Group means are presented with the standard error as the index of dispersion, except where otherwise noted. The primary analysis of interest was the comparison of the MAP during the captopril/placebo period with that during treatment with captopril/aspirin. The analysis was performed by the method of Hills and Armitage (22), which allows for separate paired testing of the effects of treatment, time, and sequence. Eicosanoid levels were analyzed by two-way (randomized blocks) analysis of variance with the SAS software package (SAS Institute, Cary, NC). Post-hoc power calculation indicated that the study had an 89% chance of detecting a 10 mm Hg change in MAP with a 5% chance of a false-positive result, on the basis of the observed standard deviation of 8 mm Hg in repeated observations in the same individuals. This was similar to the prestudy estimate.
RESULTS

We studied a total of 16 patients. Characteristics of the patients completing the study are shown in Table 1. One patient developed an asthma flare curing the initial placebo period and was withdrawn; the remainder of the patients completed the study without any significant adverse effects. As dictated by the inclusion criteria, all of the patients had mild hypertension and normal renal function. Eight patients were randomized to receive captopril/aspirin during the second treatment period and captopril/placebo during the fourth period, whereas seven received the treatments in the opposite order. Mean body weight did not vary significantly over the 8-wk period of study: 88.5 ± 15 kg after the washout before captopril/placebo, 87.9 ± 16 kg after captopril/placebo, 87.9 ± 16 kg after the washout before captopril/aspirin, and 87.7 ± 16 kg after captopril/aspirin.

The effects of the experimental maneuvers on blood and urine eicosanoids are shown in Table 2 and Figure 3. Serum TXB2, reflecting platelet thromboxane production, significantly increased during the captopril/placebo period compared with the two washout periods combined ($P = 0.049$). As shown in Figure 3, treatment with captopril/aspirin reduced serum TXB2 to an average of 53% of that observed during treatment with captopril/placebo ($P < 0.0005$). The urinary excretion of TXB2 was not significantly affected by captopril or captopril/aspirin. The excretion of this metabolite reflects thromboxane production by the kidney (23). Treatment with captopril increased urinary PGE2 ($P = 0.038$ for the comparison of the two captopril periods with the two washout periods). The urinary excretion of the prostacyclin metabolite 6-keto-PGF1a also tended to be greater during treatment with captopril ($P = 0.076$).

Figure 4 shows the mean arterial blood pressure at the end of each treatment period. Captopril/placebo significantly lowered MAP ($P < 0.05$) compared with the washout periods. However, the addition of low-dose aspirin caused no additional lowering of MAP. MAP at the end of each washout period was similar, and formal testing found no effect of the order of treatment or of time on MAP. Additionally, there was no significant linear correlation of serum TXB2 with MAP for any of the four treatment periods.

DISCUSSION

Some of the antihypertensive effects of ACEI are mediated through nonangiotensin II–dependent mechanisms. Previous studies have suggested that vasodilator prostaglandins contribute to some of these antihypertensive actions. For example, the systemic inhibition of prostaglandin production blunts the blood pressure–lowering effect of captopril (5–9). Additionally, several studies have documented in-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>53.3 ± 10.6</td>
</tr>
<tr>
<td>Sex M/4 F</td>
<td></td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>149 ± 17</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>114 ± 8</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>28.3 ± 3.7</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dL)</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

## TABLE 2. Blood and urine eicosanoids

<table>
<thead>
<tr>
<th></th>
<th>Washout</th>
<th>Captopril + Placebo</th>
<th>Washout</th>
<th>Captopril + Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum TXB2 (pg/mL)</td>
<td>553 ± 78</td>
<td>600 ± 46$^a$</td>
<td>420 ± 57</td>
<td>302 ± 36$^b$</td>
</tr>
<tr>
<td>Plasma PGE2 (ng/mL)</td>
<td>6.4 ± 1.8</td>
<td>7.0 ± 1.5</td>
<td>6.7 ± 1.3</td>
<td>7.3 ± 1.9</td>
</tr>
<tr>
<td>Plasma 6-keto-PGF1a (pg/mL)</td>
<td>86 ± 10</td>
<td>86 ± 11</td>
<td>81 ± 14</td>
<td>75 ± 11</td>
</tr>
<tr>
<td>Urine TXB2 (pg/mg of creatinine)</td>
<td>38 ± 13</td>
<td>47 ± 13</td>
<td>30 ± 4</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>Urine PGE2 (pg/mg of creatinine)</td>
<td>159 ± 20</td>
<td>307 ± 68$^c$</td>
<td>237 ± 33</td>
<td>294 ± 55$^d$</td>
</tr>
<tr>
<td>Urine 6-keto-PGF1a (pg/mg of creatinine)</td>
<td>58 ± 10</td>
<td>84 ± 26</td>
<td>41 ± 9</td>
<td>70 ± 19</td>
</tr>
</tbody>
</table>

$^a$ $P = 0.049$ versus the two washout periods combined.

$^b$ $P < 0.0005$ versus captopril/placebo.

$^c$ $P = 0.038$ for the two captopril periods versus the two washout periods.
Increases in plasma levels of metabolites of the vasodilator PGE₂ during treatment with captopril (3-5).

The effect of ACEI on thromboxane metabolism has also been addressed previously. Moore et al. found in eight hypertensive patients that a single oral dose of captopril caused a nonsignificant increase in serum TXB₂ (5). Serum TXB₂ is thought to reflect TXA₂ produced by platelets (23). In addition, Kudo et al. (24) reported an increase in the rate of the urinary excretion of thromboxane metabolites after a single oral dose of captopril. Several thromboxane metabolites are excreted in the urine. Unmetabolized TXB₂ in the urine is thought to be an index of renal thromboxane production, whereas 2,3-dinor TXB₂ is the major systemic metabolite of thromboxane in humans (23). The assay method used...
in the study of Kudo et al. potentially detects significant amounts of 2,3-dinor TXB₂. Thus, the observed increase in urinary thromboxane may have been due to increased platelet production of thromboxane.

In this study, we observed a significant increase in serum TXB₂ during treatment with captopril but no change in the urinary excretion of unmetabolized TXB₂. This suggests that, in this patient group, ACEI increases platelet thromboxane production but does not affect renal thromboxane production. Urinary PGE₂ significantly increased during treatment with captopril. Similarly, the urinary excretion of the prostacyclin metabolite 6-keto-PGF₁α tended to increase with captopril treatment. The urinary measurements may be a better indicator of total vasodilator prostaglandin formation because they reflect production over the 12 h of the urine collection rather than an instantaneous blood level, which is also subject to variability related to the vascular trauma of venipuncture. Thus, our data suggest that treatment with captopril increases the production both of vasodilator prostaglandins and of thromboxane.

We hypothesized that selective inhibition of thromboxane production would enhance the antihypertensive effect of captopril by allowing the increase in vasodilator eicosanoids to be unopposed by an increase in thromboxane production. Low-dose aspirin given in combination with captopril did significantly reduce serum TXB₂ to 53% of that observed during treatment with captopril alone; however, there was no significant effect of aspirin on blood pressure. Kudo et al. (24) reported that pretreatment with the specific thromboxane synthase inhibitor OKY-046 accentuated the antihypertensive effect of a single 50-mg dose of captopril in nine patients with essential hypertension. In that study, urinary thromboxane metabolite levels were reduced by 75% compared with treatment with captopril alone. Thus, it is possible that more complete inhibition of platelet thromboxane production is necessary to enhance the blood pressure–lowering effect of captopril or that renal thromboxane production must also be inhibited in order to observe the effect.

Higher doses of captopril may cause greater increases in prostaglandin production than we observed, making the contribution to blood pressure regulation larger relative to the angiotensin-mediated effect. Patients in this study had mild hypertension, precluding the use of higher doses of captopril. We also cannot rule out the possibility that we missed a small blood pressure effect. This study had adequate power to detect a 10 mm Hg additional effect of aspirin, but a larger study would have been required to detect a smaller blood pressure effect. For example, 43 patients would be required to be reasonably sure of detecting a difference as small as 5 mm Hg, a difference that would still be clinically significant.

The dose of aspirin used in this study was chosen on the basis of previous studies showing that 30 to 80 mg of aspirin per day inhibits platelet thromboxane production by about 95% in normal humans (13-15). It is clear from this study that 75 mg of aspirin per day is not sufficient to achieve that level of platelet thromboxane suppression in the face of treatment with captopril. This raises the possibility that higher doses of aspirin would be more beneficial in patients taking ACEI who are taking aspirin for its antithrombotic effects. However, in normal humans, aspirin substantially inhibits prostacyclin biosynthesis in doses of more than 80 mg/day (13–15). Whether the threshold for PGI₂ inhibition is altered by ACEI is unclear; however, we observed no inhibition of urinary PGI₂ metabolite excretion in patients taking captopril and low-dose aspirin.

Thromboxane may not be an important modulator of systemic blood pressure in essential hypertension. Minuz et al. (25) found no association between blood pressure and urinary thromboxane metabolite excretion in 46 patients with mild essential hypertension, whereas a weak correlation was noted for metabolites of prostacyclin. In one small study, treatment with a thromboxane synthase inhibitor in patients with mild essential hypertension did not lower blood pressure (24). Certainly, there are many hormonal and paracrine systems that affect systemic blood pressure. The manipulation of one system may cause compensatory changes in other systems that blunt the effect on blood pressure. Thus, despite our results, platelet thromboxane may have a minor role in blood pressure regulation. However, the suppression of platelet thromboxane production with aspirin is not likely to result in an enhancement of the antihypertensive effect of ACEI.

ACKNOWLEDGMENTS

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