Role of Thromboxane A2 in the Hypotensive Effect of Captopril in Essential Hypertension

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SUMMARY We have previously reported that captopril stimulates thromboxane A2 synthesis in patients with essential hypertension. In the present study, the hypotensive effects of captopril and OKY-046, a selective inhibitor of thromboxane A2 synthetase, were studied in nine patients with essential hypertension to determine whether thromboxane A2 is involved in the regulation of blood pressure. A single oral dose of OKY-046 (400 mg) decreased urinary thromboxane B2 (a stable metabolite of thromboxane A2) excretion significantly (from 113 ± 19.0 to 51.0 ± 6.1 pg/min; p<0.01) and increased urinary sodium excretion significantly (from 73.0 ± 15.3 to 113.0 ± 14.4 µEq/min; p<0.01), but no change was observed in mean arterial pressure. The administration of OKY-046 (600 mg/day) for 3 days induced a significant and sustained decrease in urinary thromboxane B2 excretion, but it did not affect the mean arterial pressure. Although captopril (50 mg) alone induced a significant increase in urinary thromboxane B2 excretion (from 91.4 ± 11.0 to 297.3 ± 30.8 pg/min; p<0.001) and a significant decrease in mean arterial pressure (from 97.0 ± 4.7 to 88.1 ± 5.1 mm Hg; p<0.01), captopril in combination with OKY-046 induced a decrease both in urinary thromboxane B2 excretion (from 70.8 ± 12.3 to 54.2 ± 14.7 pg/min; p<0.01) and in mean arterial pressure (from 105.1 ± 3.8 to 84.2 ± 3.6 mm Hg; p<0.01). Thus, the hypotensive effect of captopril was potentiated by OKY-046. OKY-046 did not affect the changes in plasma renin activity and plasma aldosterone concentration and blunted urinary prostaglandin E2 and 6-keto-prostaglandin F1α excretion in response to captopril. These results indicate that thromboxane A2 counteracts the hypotensive effect of captopril in patients with essential hypertension. (Hypertension 11: 147-152, 1988)

KEY WORDS thromboxane A2 • captopril • hypotensive mechanism • essential hypertension

THROMBOXANE A2 (TXA2) is a potent vasoconstrictor and platelet aggregating agent. Some reports suggest that increased synthesis of TXA2 is implicated in some pathological states such as angina pectoris and the development of atherosclerosis. A notable amount of TXA2 synthetase activity has been demonstrated in the human kidney. Urinary thromboxane B2 (TXB2), a stable metabolite of TXA2, is thought to reflect the renal synthesis of TXA2. Recently, it has been reported that urinary excretion of TXB2 was increased in patients with renal allograft rejection, systemic lupus erythematosus, or hepato-renal syndrome. As TXA2 is a vasoconstrictor, TXA2 is thought to be involved in the modulation of vascular tone. However, the role of TXA2 in the regulation of blood pressure and renal function has not been fully elucidated.

The renin-angiotensin and kallikrein-kinin systems are involved in the hypotensive mechanism of captopril. Recently, interest has been also focused on prostanooids. Captopril reportedly induces an increase in prostaglandin production in humans and experimental animals. Furthermore, indomethacin, an inhibitor of cyclooxygenase, has been shown to reduce the hypotensive effect of captopril in some patients with essential hypertension. A recent report from our laboratory showed that captopril, an inhibitor of angiotensin converting enzyme, induced a concomitant increase in urinary prostaglandin E (PGE) and TXB2.
Patients and Methods

The present study was conducted in five male and four female patients with essential hypertension ranging in age from 18 to 64 years old. They gave informed consent before the study. The diagnosis of essential hypertension was confirmed by history, physical and laboratory examinations, intravenous pyelography, determinations of plasma renin activity (PRA) and plasma aldosterone concentration (PAC), radioisotope renography and angiography. All patients had stopped taking antihypertensive medication at least 2 weeks before the study began and received a standard hospital diet throughout the study period.

Three different studies were performed.

Protocol 1: Effects of Short-term Administration of OKY-046

Eight patients with essential hypertension (4 men and 4 women; mean age, 40.0 ± 5.1 years) were examined to determine the short-term effects of OKY-046, (E)-3-[4-(1-imidazolylmethyl)-phenyl]-2-tropenionic acid hydrochloride monohydrate (Ono Pharmaceutical, Osaka, Japan), on systemic blood pressure. Sufficient patients were studied after an overnight fast. Experiments were performed between 0700 and 1300, during which time patients were not allowed to eat or drink. After baseline sampling of blood and urine for PRA, PAC, and urinary TXB2, a single oral dose of OKY-046 (400 mg) was administered at 0900. Blood samples were obtained 60 and 120 minutes after the administration of the drug. Blood pressure was monitored at 10-minute intervals with an automatic blood pressure recorder (Model 203, Nihon Korin, Japan) for 60 minutes in the control period and for the initial 2 hours after OKY-046 treatment. Urine samples were collected every 2 hours: 0700 to 0900, the control or baseline period; 0900 to 1100, the first 2 hours after OKY-046 administration; and 1100 to 1300, the second 2 hours after OKY-046 administration. Urine was collected and refrigerated at 4°C after each voiding, and aliquots of the urine samples were stored at −20°C until analysis.

Protocol 2: Effects of Subchronic Administration of OKY-046

Nine patients with essential hypertension (5 men and 4 women; mean age, 37.5 ± 5.1 years) were examined to determine the effect of OKY-046 on systemic blood pressure. The patients were given OKY-046, 200 mg three times daily, for 3 days, and 24-hour urine samples were collected for sodium, potassium, and TXB2 determinations. Systemic blood pressure was measured four times daily with the patients in the supine position, and values were averaged for each day.

Protocol 3: Effects of Captopril in Combination with OKY-046

The effects of captopril were examined in nine patients with essential hypertension who had previously received either placebo or OKY-046 for 3 days as described in Protocol 2. After 3 days of either placebo or OKY-046, 200 mg three times daily, patients were given captopril after an overnight fast. Studies were performed between 0700 and 1300, during which time patients were not allowed to eat or drink. The last dose of placebo or OKY-046 (400 mg) was given at 0700, and a single oral dose of captopril (50 mg) was administered at 0900. Urine and blood samples were collected in the same way as described in Protocol 1. In this protocol, each patient was studied repeatedly while taking either placebo or OKY-046.

Laboratory Procedures

All blood samples were collected on ice and spun immediately, and the plasma was separated and frozen until the time of assay. Urinary sodium and potassium were measured with a selective electrode of sodium and potassium, respectively (Technicon Stat Ion, Type II, Japan Technicon, Tokyo, Japan). PRA was determined by means of radioimmunoassay (RIA) of angiotensin I as described previously16; PAC was also determined by RIA.18

Urinary TXB2, extracted with chloroform and separated by silicic acid column chromatography, was measured by RIA as described previously.19 The cross-reactivity of anti-TXB2 serum was 100% for TXB2, 34% for 2,3-dinor TXB2, 0.36% for prostaglandin D2, 0.03% for prostaglandin F1α (PGF1α), and less than 0.01% for PGE2, PGE2, prostaglandin A1, prostaglandin B1, and 6-keto-PGF1α. Urinary PGE2 was measured by the RIA as described previously.16,20 The cross-reactivity of anti-PGE2 serum was 100% for PGE2, 10.7% for PGE1, 0.3% for prostaglandin A1, 0.04% for prostaglandin A1, and less than 0.01% for prostaglandins B1 and B2, TXB2, and 6-keto-PGF1α. In this study, the measurement of urinary PGE2 was performed only in female patients, as the concentration of PGE2 in the urine of male subjects is affected by contamination with seminal fluid.19,21 The RIA of 6-keto-PGF1α was performed on unextracted urine. The cross-reactivity of anti-6-keto-PGF1α serum was 100% for 6-keto-PGF1α, 14% for PGE1, 2% for PGE2, and PGE1, and less than 1% for TXB2 and PGE2.22 TXB2 standard, anti-TXB2 serum, and [3H]TXB2 were purchased in kit form (Model NEN-042) from New England Nuclear, Boston, MA, USA. Anti-PGE2 serum was purchased from Institute Pasteur, Paris, France. Standards of PGE2 and 6-keto-PGF1α were purchased from Sigma Chemical (St. Louis, MO, USA). Anti-serum to 6-keto-PGF1α was a gift from Dr. Michael J. Dunn, Cleveland, OH, USA. [3H]keto-PGF1α and [3H]PGE2 were purchased from New England Nuclear.
Statistics
The mean arterial pressure (MAP) was calculated from systolic and diastolic blood pressures in the conventional way. Group means are presented with the standard error of the mean as the index of dispersion. Statistical probability was evaluated using the paired *t* test.

Results

Short-term Effects of OKY-046 (Protocol 1)
The administration of OKY-046 induced a significant and sustained decrease in urinary TXB2 excretion when compared with the control value (Table 1). It also induced a significant increase in urine volume and urinary sodium or potassium excretion. However, MAP did not change significantly (106.7 ± 2.6 mm Hg at control, 103.1 ± 2.4 mm Hg at 60 minutes, and 102.1 ± 2.0 mm Hg at 120 minutes; Figure 1).

Subchronic Effects of OKY-046 (Protocol 2)
The administration of OKY-046 (600 mg/day) for 3 days induced a significant and sustained decrease in urinary TXB2 excretion when compared with the control value (Table 2). MAP, urinary potassium excretion, and urine volume did not change significantly, although a slight but insignificant increase was observed in urinary sodium excretion during the administration of OKY-046 on Day 1.

Effects of Captopril in Combination with OKY-046 (Protocol 3)
Captopril administration in combination with OKY-046 induced a significant decrease in MAP (from 105.1 ± 3.8 to 84.2 ± 3.6 mm Hg; control vs 60 minutes; *p*<0.01), as did administration of captopril with placebo (from 97.0 ± 4.7 to 88.1 ± 5.1 mm Hg; control vs 60 minutes; *p*<0.01; see Figure 1). When measured as a change of MAP the administration of OKY-046 clearly potentiated the hypotensive effect of captopril (Figure 2).

As shown in Table 3, administration of captopril alone induced a significant increase in urinary TXB2 excretion (from 91.4 ± 11.0 to 297.3 ± 30.8 pg/min; control urine vs first posttreatment urine sample; *p*<0.001). It also induced increases in urine volume, urinary sodium, PGE2, and 6-keto-PGF1α excretion. On the other hand, treatment with OKY-046 completely inhibited the captopril-induced increase in urinary TXB2 excretion (see Table 3). The responses in PRA and PAC to captopril did not differ significantly between the patients given OKY-046 and those given placebo. Prior administration of OKY-046 did not change significantly the effect of captopril on PRA and PAC, whereas it blunted increases in urinary PGE2 and 6-keto-PGF1α excretion in these patients. To avoid the problem of contamination with seminal fluid in male subjects, urinary PGE2 excretion was measured only in 4 female subjects. Thus, potentiation of the hypotensive effect of captopril by OKY-046 was accompanied exclusively by the inhibition of captopril-induced TXA2 generation.

Discussion
In the present study in patients with essential hypertension, urinary excretion of TXB2 was significantly reduced by either short-term or subchronic administration of OKY-046. However, the MAP was not changed significantly in these experiments. Similar results have been reported using other selective inhibitors of TXA2 synthetase, such as dazoxiben or OKY-1581. It has been also reported that there was no consistent difference in the basal excretion of TXB2 between normal subjects and hypertensive patients. Thus, under basal conditions in which renal TXA2 synthesis is not increased, TXA2 may not play an important role in the regulation of blood pressure in patients with essential hypertension.

TXA2, and prostaglandin I2 (PGI2) may exert opposite effects on platelet aggregation and vascular resistance. The balance between these two compounds has been suggested as one of the factors that determine platelet reactivity, endothelial thromboresistance, and vascular tone. Therefore, much attention has been focused on the TXA2/PGI2 balance. An inhibitor of

![Figure 1. Changes in MAP after the administration of 400 mg of OKY-046 alone (A), 50 mg of captopril alone (O), or 50 mg of captopril combined with a 4-day pretreatment with OKY-046 (600 mg/day for 3 days and 400 mg/day for 1 day; (●). Results are means ± SEM. Asterisk indicates significant difference (*p*<0.01) compared with respective control levels.]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>0-2 hr posttreatment</th>
<th>2-4 hr posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTXB2V (pg/min)</td>
<td>113.0 ± 19.0</td>
<td>51.0 ± 6.1*</td>
<td>32.0 ± 5.9*</td>
</tr>
<tr>
<td>UKV (μEq/min)</td>
<td>73.0 ± 15.3</td>
<td>113.0 ± 14.4*</td>
<td>79.0 ± 11.7</td>
</tr>
<tr>
<td>UKV (μEq/min)</td>
<td>35.0 ± 6.9</td>
<td>49.0 ± 3.2*</td>
<td>34.0 ± 3.8</td>
</tr>
<tr>
<td>UV (ml/min)</td>
<td>0.81 ± 0.20</td>
<td>1.49 ± 0.29*</td>
<td>0.47 ± 0.06</td>
</tr>
</tbody>
</table>

Results are means ± SEM of eight subjects. UTXB2V = urinary thromboxane B2 excretion; UKV = urinary potassium excretion; UV = urinary volume. *p<0.01, compared with control urine value.
TABLE 2. Subchronic Effects of OKY-046

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control day</th>
<th>OKY-046, 600 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>112.0 ± 1.78</td>
<td>108.6 ± 2.06</td>
</tr>
<tr>
<td>UTXB2 V (ng/day)</td>
<td>165.7 ± 17.8</td>
<td>52.8 ± 5.4*</td>
</tr>
<tr>
<td>UNa V (mg/day)</td>
<td>190.0 ± 23.0</td>
<td>235.0 ± 36.0</td>
</tr>
<tr>
<td>UKV (mEq/day)</td>
<td>32.7 ± 4.7</td>
<td>42.8 ± 4.4</td>
</tr>
<tr>
<td>UV (ml/day)</td>
<td>1491.0 ± 132.0</td>
<td>108.6 ± 2.06</td>
</tr>
</tbody>
</table>

Results are means ± SEM of nine subjects. OKY-046 (600 mg) was administered on Days 1, 2, and 3. UTXB2 V = urinary TXB2 excretion, UNa V = urinary sodium excretion; UKV = urinary potassium excretion; UV = urinary volume. *p < 0.001, compared with control day value.

TXA2 synthetase may not only inhibit the production of TXA2 but also preserve or even enhance the production of PGI2. However, in the present study, OKY-046 did not increase urinary 6-keto-PGF1α or PGE2 excretion. In accord with our results, other inhibitors of TXA2 synthetase, such as OKY-1581 or dazoxiben, have been reported to decrease PGI2 production.28,29

In the present study, we confirmed our previous findings that the angiotensin converting enzyme inhibitor captopril increases urinary excretion of PGE2 and TXB2. OKY-046 blocked the captopril-induced augmentation in urinary TXB2 excretion. Furthermore, OKY-046 significantly potentiated the hypotensive effect of captopril in patients with essential hypertension. This potentiation of the hypotensive effect of captopril was accompanied by a reduction in urinary TXB2 excretion. Enhanced vasodilator prostaglandins, such as PGE2 and PGI2, are thought to play a role in the hypotensive effect of captopril.13,14,16

In the present study, however, pretreatment with OKY-046 blunted the captopril-induced PGE2 and 6-keto-PGF1α production.

TABLE 3. Effect of Captopril in Essential Hypertensive Patients With or Without OKY-046 Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control + placebo</th>
<th>Captopril + OKY-046</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–2 hr posttreatment</td>
<td>2–4 hr posttreatment</td>
</tr>
<tr>
<td></td>
<td>Control + placebo</td>
<td>Captopril + OKY-046</td>
</tr>
<tr>
<td>UTXB2 V (pg/min)</td>
<td>91.4 ± 11.0 297.3 ± 30.8*</td>
<td>178.3 ± 31.9</td>
</tr>
<tr>
<td>UPG12 V (pg/min)</td>
<td>91.4 ± 16.9 198.8 ± 33.9</td>
<td>104.0 ± 10.2</td>
</tr>
<tr>
<td>U6_keto-PGF1α V (pg/min)</td>
<td>182.5 ± 13.8 336.6 ± 64.7</td>
<td>273.8 ± 62.5</td>
</tr>
<tr>
<td>UV (μl/min)</td>
<td>0.62 ± 0.07 1.94 ± 0.28*</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td>UNa V (μEq/min)</td>
<td>98.2 ± 13.3 230.6 ± 22.6*</td>
<td>126.4 ± 13.7</td>
</tr>
<tr>
<td></td>
<td>Control 60 min 120 min</td>
<td>Control 60 min 120 min</td>
</tr>
<tr>
<td>PRA (ng Ang I/ml/hr)</td>
<td>1.44 ± 0.42 9.34 ± 3.17</td>
<td>8.89 ± 2.96</td>
</tr>
<tr>
<td>PAC (ng/dl)</td>
<td>6.91 ± 1.00 4.81 ± 0.99</td>
<td>4.62 ± 1.29</td>
</tr>
</tbody>
</table>

Results are means ± SEM of nine patients, except for UPG12 V, where results are means ± SEM of four female patients. UTXB2 V = urinary TXB2 excretion; UPG12 V = urinary PGE2 excretion; U6_keto-PGF1α V = urinary 6-keto-PGF1α excretion; UV = urinary volume, UNa V = urinary sodium excretion; Ang I = angiotensin I; PAC = plasma aldosterone concentration.

*p < 0.001, †p < 0.02, ‡p < 0.05, compared with respective control values.

$^p < 0.01, $^p < 0.001, compared with respective captopril + placebo values.
tion but did not affect PRA and PAC. Thus, the OKY-046-induced potentiation of the hypotensive effect of captopril may be mediated by the inhibition of capto- 

pril-induced TXA2 synthesis.

It is interesting that the TXA2 synthetase inhibitor exerted its effect on blood pressure not under basal conditions but when the renal TXA2 synthesis was increased by captopril treatment. It has been reported in experimental animals that TXA2 is not synthesized in the kidney under physiological conditions, but its synthesis is induced in certain pathological states of the kidney, such as hydronephrosis, renal vein constric-

tion, or acute renal failure. These findings suggest that TXA2 may play a role in the regulation of blood pressure only under certain conditions in which TXA2 production is increased.

Recently, a thromboxane synthetase inhibitor, imi-
dazole, was reported to increase urinary sodium excretion in rats. In the present study, the short-term administration of OKY-046 induced a significant increase in urinary sodium excretion in patients with essential hypertension. The natriuresis was accom-

panied by the reduction of urinary TXB2 excretion. The subchronic administration of OKY-046 also induced a slight but insignificant increase in urinary sodium excretion and a weak diuresis. To clarify the mechanisms of the effects of TXA2 synthetase inhibitor on renal excretory function, further experiments are needed.

As discussed, the potentiation of the hypotensive effect of captopril with OKY-046 is supposedly due to the inhibition of TXA2 production in the kidney. How-

ever, we have not examined the effect of captopril on TXA2 synthesis in extrarenal tissues. Recently, it has been reported that TXA2 is also synthesized in human vascular wall. There is a possibility that captopril may also stimulate the production of TXA2 in vascular wall, which eventually reduces the hypotensive effect of the converting enzyme inhibitor. Thus, it may be more likely that the potentiation of the hypotensive effect of captopril with OKY-046 is mediated by the inhibition of TXA2 synthesis, not only in the kidney but also in the systemic vasculature.

In conclusion, our results suggest that TXA2 may be of negligible importance for the regulation of blood pressure under basal conditions. However, certain sit-

uations in which TXA2 production is increased by some disease or drugs such as converting enzyme inhibitors, TXA2 may play a role as a potent vasocon-

strictor in the regulation of blood pressure in humans.

Acknowledgments

We are grateful to Dr. Michael J. Dunn of Case Western Reserve University (Cleveland, OH, USA) for the gift of anti-6-keto-PGF1α serum. We are also grateful for the excellent technical assistance of Kaori Matsuura, Keiko Shinoishi, and Mayumi Nakayama and the secretarial assistance of Junko Okazaki.

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