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

Kei Kudo, Keishi Abe, Satoru Chiba, Makito Sato, Minoru Yasujima, Masahiro Kohzuki, Ken Omata, Masaya Tanno, Kazuo Tsunoda, and Kaoru Yoshinaga

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
excretion in patients with essential hypertension.\textsuperscript{17} Thus, enhanced production of prostaglandins and TXA\textsubscript{2} may influence (i.e., either potentiate or blunt) the hypotensive effect of captopril.

In the present study, we investigated the effect of a selective inhibitor of TXA\textsubscript{2} synthetase, OKY-046, on blood pressure in patients with essential hypertension receiving captopril in an attempt to elucidate the role of TXA\textsubscript{2} in the hypotensive mechanism of captopril.

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-unidazolylmethyl)-phenyl]-2-troponic acid hydrochloride monohydrate (Ono Pharmaceutical, Osaka, Japan), on systemic blood pressure. Supine 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 TXB\textsubscript{2}, 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 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 TXB\textsubscript{2} 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 studied supine 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 previously; PAC was also determined by RIA.\textsuperscript{18} Urinary TXB\textsubscript{2}, extracted with chloroform and separated by silicic acid column chromatography, was measured by RIA as described previously.\textsuperscript{19} The cross-reactivity of anti-TXB\textsubscript{2} serum was 100% for TXB\textsubscript{2}, 34% for 2,3-dinor TXB\textsubscript{2}, 0.36% for prostaglandin D\textsubscript{2}, 0.03% for prostaglandin F\textsubscript{1α} (PGF\textsubscript{1α}), and less than 0.01% for PGE\textsubscript{1}, PGE\textsubscript{2}, prostaglandin A\textsubscript{1}, prostaglandin B\textsubscript{2}, and 6-keto-PGF\textsubscript{1α}. Urinary PGE\textsubscript{2} was measured by the RIA as described previously.\textsuperscript{18} The cross-reactivity of anti-PGE\textsubscript{2} serum was 100% for PGE\textsubscript{2}, 10.7% for PGE\textsubscript{1}, 0.3% for prostaglandin A\textsubscript{2}, 0.04% for prostaglandin A\textsubscript{1}, and less than 0.01% for prostaglandins B\textsubscript{1} and B\textsubscript{2}, TXB\textsubscript{2}, and 6-keto-PGF\textsubscript{1α}. In this study, the measurement of urinary PGE\textsubscript{2} was performed only in female patients, as the concentration of PGE\textsubscript{2} in the urine of male subjects is affected by contamination with seminal fluid.\textsuperscript{18,19} The RIA of 6-keto-PGF\textsubscript{1α} was performed on unextracted urine. The cross-reactivity of anti-6-keto-PGF\textsubscript{1α} serum was 100% for 6-keto-PGF\textsubscript{1α}, 14% for PGF\textsubscript{1α}, 2% for PGE\textsubscript{2}, PGF\textsubscript{1α}, and less than 1% for TXB\textsubscript{2} and TXB\textsubscript{2}. TXB\textsubscript{2} standard, anti-TXB\textsubscript{2} serum, and [\textsuperscript{3}H]TXB\textsubscript{2} were purchased in kit form (Model NEN-042) from New England Nuclear, Boston, MA, USA. Anti-PGE\textsubscript{2} serum was purchased from Institute Pasteur, Paris, France. Standards of PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1α} were purchased from Sigma Chemical (St. Louis, MO, USA). Anti-serum to 6-keto-PGF\textsubscript{1α} was a gift from Dr. Michael J. Dunn, Cleveland, OH, USA. [\textsuperscript{3}H]6-keto-PGF\textsubscript{1α} and [\textsuperscript{3}H]PGE\textsubscript{2} 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 (600 mg/day for 3 days and 400 mg/day for 1 day; •). 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 TXA₂ synthesis such as OKY-046 is expected to be effective in patients with essential hypertension.

Table 1: Short-term Effects of OKY-046 Alone

<table>
<thead>
<tr>
<th>Variable</th>
<th>Urine sample</th>
<th>0-2 hr posttreatment</th>
<th>2-4 hr posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>U_TXB2V (pg/min)</td>
<td>Control</td>
<td>113.0 ± 19.0</td>
<td>51.0 ± 6.1*</td>
</tr>
<tr>
<td>U_NaV (µEq/min)</td>
<td>Control</td>
<td>73.0 ± 15.3</td>
<td>113.0 ± 14.4*</td>
</tr>
<tr>
<td>U_KV (µEq/min)</td>
<td>Control</td>
<td>35.0 ± 6.9</td>
<td>49.0 ± 3.2*</td>
</tr>
<tr>
<td>UV (ml/min)</td>
<td>Control</td>
<td>0.81 ± 0.20</td>
<td>1.49 ± 0.29*</td>
</tr>
</tbody>
</table>

Results are means ± SEM of eight subjects. *p<0.01, compared with control urine value.

Figure 1. Changes in MAP after the administration of 400 mg of OKY-046 alone (●), 50 mg of captopril alone (○), 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 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></td>
<td>Day 1</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>112.0±1.78</td>
<td>108.6±2.06</td>
</tr>
<tr>
<td>UTXB_2 V (ng/day)</td>
<td>165.7±17.8</td>
<td>52.8±5.4*</td>
</tr>
<tr>
<td>UNa V (mEq/day)</td>
<td>190.0±23.0</td>
<td>235.0±36.0</td>
</tr>
<tr>
<td>UK V (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>
<tr>
<td></td>
<td></td>
<td>1491.0±132.0</td>
</tr>
</tbody>
</table>

Results are means ± SEM of nine subjects. OKY-046 (600 mg) was administered on Days 1, 2, and 3. UTXB_2 V = urinary TXB_2 excretion; UNa V = urinary sodium excretion; UK V = urinary potassium excretion; UV = urinary volume. *p<0.001, compared with control day value.

FIGURE 2. Changes in MAP after the administration of 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. Single (p<0.02) and double (p<0.01) asterisks indicate significant difference compared with captopril treatment alone.

TXA_2 synthetase may not only inhibit the production of TXA_2, but also preserve or even enhance the production of PGI_2. However, in the present study, OKY-046 did not increase urinary 6-keto-PGF_1α or PGE_2 excretion. In accord with our results, other inhibitors of TXA_2 synthetase, such as OKY-1581 or dazoxiben, have been reported to decrease PGI_2 production.28, 29

In the present study, we confirmed our previous findings that the angiotensin converting enzyme inhibitor captopril increases urinary excretion of PGE_2 and TXB_2. OKY-046 blocked the captopril-induced augmentation in urinary TXB_2 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 TXB_2 excretion. Enhanced vasodilator prostaglandins, such as PGE_2 and PGI_2, 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 PGE_2 and 6-keto-PGF_1α production.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Captopril + placebo</th>
<th>Captopril + OKY-046</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–2 hr 2–4 hr posttreatment</td>
<td>0–2 hr 2–4 hr posttreatment</td>
</tr>
<tr>
<td>UTXB_2 V (pg/min)</td>
<td>91.4±11.0 297.3±30.8* 178.3±31.9</td>
<td>70.8±12.3 54.2±14.7t 40.2±6.7†§</td>
</tr>
<tr>
<td>UPG Eα V (pg/min)</td>
<td>91.4±16.9 198.8±33.9t 104.0±10.2</td>
<td>94.4±11.1 142.2±33.4 92.0±17.7</td>
</tr>
<tr>
<td>U6_keto_PGF_1α V (pg/min)</td>
<td>182.5±13.8 336.6±64.7 273.8±62.5</td>
<td>219.3±28.6 210.5±42.5 239.6±53.3</td>
</tr>
<tr>
<td>UV (μl/min)</td>
<td>0.62±0.07 1.94±0.28* 0.70±0.06</td>
<td>1.69±0.34§ 2.70±0.37§ 0.90±0.14</td>
</tr>
<tr>
<td>UNa V (μEq/min)</td>
<td>98.2±13.3 230.6±22.6* 126.4±13.7</td>
<td>172.8±20.0l 226.8±29.6l 142.1±21.1</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 UPG Eα V, where results are means ± SEM of four female patients. UTXB_2 V = urinary TXB_2 excretion; UPG Eα V = urinary PGE_2 excretion; U6_keto_PGF_1α V = urinary 6-keto-PGF_1α 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 captopril-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 hydropnephrosis, renal vein constriction, 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, imidazole, 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 accompanied 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. However, 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 situations in which TXA2 production is increased by some disease or drugs such as converting enzyme inhibitors, TXA2 may play a role as a potent vasoconstrictor in the regulation of blood pressure in humans.

Acknowledgments

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