Effects of Cicletanine on Prostaglandin I₂ and E₂ Levels in Patients With Essential Hypertension
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Cicletanine is a new antihypertensive drug that seems to stimulate the synthesis of prostaglandin (PG) I₂. However, there is little evidence that cicletanine increases the level of PGI₂ in the systemic blood of human subjects long-term. To investigate the antihypertensive mechanism of cicletanine, we measured serially the systemic blood pressure and the levels of both 6-keto-PGF₁α (a stable metabolite of PGI₂) and PGE₂ in plasma and urine after administration of cicletanine. Nine patients with essential hypertension on a diet with sodium intake of 120 mEq/day took 100 mg of the drug orally daily every day for 1 week. Systemic blood pressure was measured hourly for 24 h on day 7 of the control period and on days 1 and 7 of the cicletanine period. The two PGs of interest were extracted, purified by high pressure liquid chromatography, and measured by radioimmunoassay. Cicletanine decreased blood pressure without reflexial tachycardia. The plasma levels of 6-keto-PGF₁α were slightly, but significantly, higher at 3 h after the administration of cicletanine on both days 1 and 7 of administration (on day 1, 3.88 ± 1.44 pg/mL and on day 7, 4.07 ± 0.76, means ± SD, both P < .05 v before administration on day 1) than before administration on day 1 (3.21 ± 1.25 pg/mL). Plasma PGE₂ was higher before and at 3 h after administration on day 7 than at 12 noon on day 7 of the control period. Cicletanine increased the urinary excretion of the two PGs; the increased PG levels partly account for the increased natriuresis in the first 3 days. The antihypertensive effects of cicletanine taken for 1 week were based on natriuresis caused by increased systemic synthesis of the vasodilator PGI₂ and partly by the increased renal synthesis of PGI₂ and PGE₂. Am J Hypertens 1997;10:750–755 © 1997 American Journal of Hypertension, Ltd.

The antihypertensive action of cicletanine may involve natriuresis, vasodilatation by stimulation of prostaglandin (PG) I₂ synthesis, and inhibition of sympathetic nerve activity. Measurement of plasma levels of 6-keto-PGF₁α, a stable metabolite of PGI₂, in the clinical laboratory has been difficult, so few details are known about the effects of clinical doses of cicletanine on PGI₂ synthesis in patients with hypertension. We recently found that cicletanine (100 mg orally) increases the...
plasma level of 6-keto-PGF$_{1\alpha}$ at 3 and 6 h after administration in patients with essential hypertension. Plasma levels of 6-keto-PGF$_{1\alpha}$ can be measured with accuracy by radioimmunoassay after extraction and purification with high pressure liquid chromatography (HPLC). The increase in PG$_I_2$ synthesis may contribute to the antihypertensive effect on the short term. Here, we extended our investigation to the effects during 7 days of cicletanine administration on PG$_I_2$ and PGE$_2$ synthesis by measuring the plasma and urinary levels of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ in the same patients with essential hypertension as were previously studied, as well as by recording changes in blood pressure and urinary sodium excretion.

**PATIENTS AND METHODS**

**Patients** The subjects were nine patients admitted to the National Cardiovascular Center with the diagnosis of essential hypertension. They were the same patients as in our previous report. All patients had a systolic blood pressure of 160 mm Hg or higher, a diastolic pressure of 90 mm Hg or higher, or both when blood pressure was measured before admission. There were four men and five women aged 29 to 68 years, with a mean age of 45 years. They were 160 ± 12 cm tall (mean ± SD) and weighed 55 ± 6 kg. Patients with diabetes, cardiovascular disease, or a creatinine clearance of 70 mL/min or less were excluded. All patients gave informed consent to the study.

**Study Protocol** All medication was stopped for at least 1 week before the study began. Throughout the 2 weeks of the study, all patients had a dietary sodium intake of about 120 mEq/day. The first 7 days of the study was the control period, and no medication was given. During the next 7 days, cicletanine was given at the dose of 100 mg/day orally. Blood pressure and pressure in the left arm were measured by specially instructed nurses, with the patient at rest supine at 7 am, noon, 5 pm, and 9 pm every day in the 2-week study period. The results obtained each day were averaged. Daily urinary volume and sodium excretion were measured every day in the study period. Blood was sampled at 9 am (before administration, if during the administration period) and at noon (3 h after administration) on day 7 of the control period and on days 1 and 7 of cicletanine administration. Blood was drawn by puncture of an antecubital vein without stasis through a 21-gauge needle. The initial 2 mL of blood was discarded, and then 15 mL was taken. Blood samples were immediately placed in ice-cooled tubes containing ethylenediamine tetraacetic acid-2Na (1 mg/mL) and the contents were centrifuged at 4°C for 10 min at 3,000 g. Plasma samples were immediately frozen and stored at −70°C until assayed. On day 7 of the control period and days 1 and 7 of cicletanine administration, systemic blood pressure and the pulse in the left arm were measured with the patient at rest supine twice hourly for the 24 h of the study with an automatic ultrasound sphygmomanometer (BP-103, Nippon Colin Co., Aichi, Japan). The data obtained each hour were averaged. None of the patients complained of any side effects of cicletanine.

**Measurement of Prostaglandins** The levels of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ in plasma and urine were measured by radioimmunoassay after the extraction and purification of these compounds as described previously. In brief, 5 mL of plasma or urine was acidified with 4 volumes of 50 mmol/L citric acid and the mixture was put on a Bond-Elut C$_{18}$ column (500 mg, Analytichem International, Harbor City, CA). The column was washed three times with 3 mL of distilled water, 3 mL of 10% methanol, and 3 mL of cyclohexane, in that order. The fraction containing PGs was eluted with 2.5 mL of ethyl acetate and the eluate was evaporated to dryness under a stream of nitrogen. The dried sample was dissolved in 500 mL of solution A, which was a 20:80:0.1 mixture of acetonitrile, water, and trifluoroacetic acid. A 500-mL portion was injected into a reverse-phase column for HPLC. The HPLC system consisted of an autosampler (SIL-6B), two HPLC pumps (LC-9A), a system controller (SCL-6B), precolumn (TSK gel ODS-80, 4.6 × 10 mm), separation column (TSK gel ODS-80, 4.6 × 150 mm), and autofraction collector (SF-2120, Advantec Corp., Tokyo, Japan). The HPLC columns were obtained from Tosoh (Tokyo) and other components were obtained from Shimadzu (Kyoto, Japan). For chromatography, the column was equilibrated with solution A at the flow rate of 1.0 mL/min at 24°C. After injection of the sample, a linear gradient of 20% to 50% acetonitrile was used for the next 40 min. Isocratic elution with 100% solution B, which was a 100:0.1 mixture of acetonitrile and trifluoroacetic acid, was done for 20 min to wash the column. The column was equilibrated again with solution A before the injection of the next sample. Preliminary experiments showed that [3H]6-keto-PGF$_{1\alpha}$ and [3H]PGE$_2$ were eluted at about 18 and 24 min, respectively, after the injection, so the eluate was collected every 30 sec from 16.5 to 26.5 min after injection. After counting of the radioactivity of each tube in a liquid scintillation counter (LKB 1217), two or three samples with the highest radioactivity were combined, evaporated to dryness, and assayed. Concentrations of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ were measured with radioimmunoassay kits for 6-keto PGF$_{1\alpha}$ (125I) and PGE$_2$ (125I), (New England Nuclear Corp., Boston, MA). The radioactivity of 125I was counted in a gamma counter (model 5550, Packard). The values were not corrected for recovery of PGE$_2$ or 6-keto-PGF$_{1\alpha}$ for reasons described previously.
Statistics  Plasma levels of PGE2 and 6-keto-PGF$_{1\alpha}$ were assessed with repeated measures analysis via multivariate analysis of variance. Values for blood pressure, pulse rate, daily urinary volume, and daily urinary sodium excretion also were compared by repeated measures analysis via multivariate analysis of variance. Urinary excretion of PGs differed for men and women. Therefore, we used the percent change of each value to evaluate the effects of cicletanine on 24 h urinary excretion of the PGs. These percent changes were compared by one-factor analysis of variance. All results are expressed as means ± SD, as medians with 10th and 90th percentiles, or both. Differences with probability levels < .05 were considered to be statistically significant.

The statistical analysis was done with Stat View IV and Super ANOVA software (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Systemic blood pressure was decreased by cicletanine. The decreases in systolic and diastolic blood pressures were significant on day 1 of cicletanine administration, and pressures remained low for the next 4 days, although not as low as on day 1 (Figure 1). The systolic and diastolic blood pressures decreased more on days 6 and 7 of administration. The pulse rate was not changed by cicletanine. On day 7, blood pressure at 9 AM before administration was lower than on control day 7 (Figure 2).

Plasma levels of PGs are shown in Table 1. The 6-keto-PGF$_{1\alpha}$ level in plasma was higher at 3 h after the first administration than immediately before. The plasma 6-keto-PGF$_{1\alpha}$ level before administration on day 7 was not different from the value before the first administration, but was higher at 3 h after administration of cicletanine than on control day 7 or at noon of day 1. The plasma levels of PGE$_2$ were not significantly changed at 3 h after the first administration of cicletanine. The plasma PGE$_2$ level was higher both before and 3 h after administration on day 7 than on control day 7.

Urinary excretion of 6-keto-PGF$_{1\alpha}$ increased by 78% in the first day of drug administration and stayed higher throughout the administration period (Figure 3). Changes in the urinary excretion of PGE$_2$ paralleled those in the excretion of 6-keto-PGF$_{1\alpha}$. The urinary volume tended to increase on the first day of cicletanine administration (Figure 4, top). Urinary sodium excretion was unchanged through the control period, remaining in the range of 100 to 110 mEq/day (Figure 4, bottom). Cicletanine caused natriuresis in the first 3 days. Sodium excretion on day 4 of cicletanine had returned to the control range.

DISCUSSION

Cicletanine had an antihypertensive effect in hypertensive patients, causing natriuresis but little if any diuresis during the first 3 days of the administration. Cicletanine increased the urinary excretion of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ during the 7-day administration period, and increased the plasma levels of 6-keto-PGF$_{1\alpha}$ at 3 h after the administration on both days 1 and 7 of administration.

FIGURE 1.  Serial daily values for blood pressure (▲ and ▼) and pulse rate (●) during the end of the control period (days 4 to 7) and cicletanine (Ci) administration on days 1 to 7. Results are expressed as means ± SD. The pulse rate was not changed by cicletanine.

FIGURE 2.  Changes in blood pressure on day 7 of the control period (△ and ◇), on day 1 of cicletanine administration (▲ and ▼), and on day 7 of administration (●). During the administration period, the measurements at 9 AM were made immediately before administration. Results are expressed as means ± SD. Three hours after the administration of cicletanine, systolic blood pressures were significantly lower than the values at the same time during day 7 of the control period.

FIGURE 3.  Changes in urinary excretion of 6-keto-PGF$_{1\alpha}$ before administration on day 1 of drug administration (▲ and ▼) and on day 7 of the administration (●). Results are expressed as means ± SD. The urinary excretion of 6-keto-PGF$_{1\alpha}$ increased by 78% in the first day of drug administration and stayed higher throughout the administration period.
Hemodynamics Our results concerning the antihypertensive action of cicletanine in patients with hypertension are compatible with those of previous reports.1,10 Tsunoda et al3 reported that in healthy men, blood pressure is not changed by the administration of 200 mg of cicletanine although diuresis and natriuresis both occur. The different responses to cicletanine of hypertensive patients and healthy men may involve the pathogenesis of essential hypertension, inasmuch as urinary PGE2 decreases after 2,000 cc of saline (308 mEq of sodium) is infused into normotensive men over a 4-h period, but their blood pressure does not rise.11

Plasma 6-Keto-PGF1\(_\alpha\) and PGE2 The accuracy of the method we used to measure plasma 6-keto-PGF1\(_\alpha\) was reported earlier.7 PGI2 is synthesized mostly in vascular walls, especially the endothelium of arteries and arterioles, and is spontaneously hydrolyzed to 6-keto-PGF1\(_\alpha\), which is biologically inactive. Purification by HPLC is essential for accuracy in the radioimmunoassay of plasma levels of 6-keto-PGF1\(_\alpha\) because these levels are low,12 and because nonspecific immunoreactive substances interfere with the measurement.

<table>
<thead>
<tr>
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<th>6-Keto-PGF1(_\alpha) (pg/mL)</th>
<th>PGE2 (pg/mL)</th>
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<tbody>
<tr>
<td>Control day 7, 9 AM</td>
<td>3.61 ± 1.14</td>
<td>3.43 (2.52, 5.08)</td>
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<tr>
<td>Noon</td>
<td>3.36 ± 1.03</td>
<td>3.53 (1.93, 4.38)</td>
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<tr>
<td>Cicletanine day 1, before 3 h after</td>
<td>3.21 ± 1.25</td>
<td>3.42 (1.40, 4.62)</td>
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<tr>
<td>Cicletanine day 7, before 3 h after</td>
<td>3.65 ± 0.76</td>
<td>4.36 (1.78, 5.28)</td>
</tr>
<tr>
<td></td>
<td>4.07 ± 0.76*‡</td>
<td>3.82 (3.35, 5.07)</td>
</tr>
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Results (all, \(n = 9\)) are expressed as means ± SD on the left and medians with 10th and 90th percentiles on the right.

* \(P < .05\) v values at noon on control day 7; § \(P < .05\) v values at 9 AM on control day 7; and † \(P < .05\) and ‡ \(P < .01\) v values before administration of cicletanine on day 1.

FIGURE 3. Percent changes in urinary 6-keto-prostaglandin (PG) F1α (black bars) and PGE2 (white bars). Urinary excretion of 6-keto-PGF1\(_\alpha\) and PGE2 was higher on the first day of administration than the control value, and remained high on days 3 and 7.

FIGURE 4. Urinary sodium excretion (top) and urinary volume (bottom) during days 4 to 7 of the control period and days 1 to 7 of cicletanine period. Cicletanine caused natriuresis in the first 3 days, without causing diuresis.
Here we found that the plasma level of 6-keto-PGF1α was slightly but significantly higher at 3 h after the administration of cicletanine on day 7. How cicletanine increases PGI2 synthesis is unknown, but the increase may contribute to the antihypertensive effect of this drug given long-term.

Plasma levels of PGE2 did not increase 3 h after the first administration but were high both before and 3 h after administration on day 7. The increase on day 7 might reflect a large increase in renal PGE2 synthesis, because PGE2 is synthesized mainly in the kidneys and is metabolized rapidly during circulation through the lungs.13,14

Urinary Excretion of 6-Keto-PGF1α, PGE2, and Sodium
Cicletanine increases the urinary excretion of 6-keto-PGF1α and PGE2 in humans3,10 and rats.4,6,15 We saw an increase by about 80% in urinary excretion of 6-keto-PGF1α and PGE2 in the first 24 h. This level of excretion, when tested on days 3 and 7, was found to be maintained. Therefore cicletanine stimulated the renal synthesis of PGI2 and PGE2, as the urinary excretion of these PGs reflects mainly their renal and prostatic synthesis. Cicletanine decreases salt-induced hypertension in spontaneously hypertensive rats and increases their urinary excretion of PGE2.4 The mean blood pressure of the rats changes inversely from changes in PGE2 excretion, indicating that potentiation of renal PGE2 synthesis is a part of the antihypertensive mechanism of cicletanine. PGE2, the principal intermediary of the renal PG system, probably interferes with the stimulation of adenylate cyclase by vasopressin and thereby decreases vasopressin-stimulated water transport. Cicletanine causes natriuresis with diuresis in healthy male volunteers at doses of 200 mg/day3 and in patients with essential hypertension.10,16 The doses that cause natriuresis and diuresis in rats have been reported.17,18 Buchholz et al17 reported that cicletanine is natriuretic in conscious hydrated normotensive rats at a smaller dose (3 mg/kg) and is metabolized rapidly during circulation through the lungs.19

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