Comparison of the Antihypertensive Effects of the New Angiotensin II (AT1) Receptor Antagonist Candesartan Cilexetil (TCV-116) and the Angiotensin Converting Enzyme Inhibitor Enalapril in Rats

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Antihypertensive effects of an angiotensin (Ang) II receptor antagonist, candesartan cilexetil (TCV-116), were compared with those of an angiotensin converting enzyme (ACE) inhibitor, enalapril, in spontaneously hypertensive rats (SHR), 2-kidney, 1-clip hypertensive rats (2K, 1C-HR) and 1-kidney, 1-clip hypertensive rats (1K, 1C-HR). CV-11974, the active form of TCV-116, had no inhibitory activity for plasma ACE. In rats, TCV-116 inhibited the pressor responses to Ang I, Ang II, and Ang III without an effect on the bradykinin (BK)-induced depressor response. Enalapril inhibited only the Ang I-response and potentiated the BK-response. In SHR, the antihypertensive effect of TCV-116 (10 mg/kg) was larger than the maximum antihypertensive effect of enalapril and was not intensified by combination with enalapril. Administration of CV-11974 potentiated the maximum antihypertensive effect of enalapril. Although both agents reduced blood pressure in 2K, 1C-HR, only TCV-116 had a marked antihypertensive effect in 1K, 1C-HR. These findings indicate that TCV-116 is more effective than enalapril in reducing blood pressure in SHR and 1K, 1C-HR, and that the BK- and/or prostaglandin-potentiating effect of enalapril contributes little to its antihypertensive mechanism in SHR. (*Hypertens Res* 1996; 19: 75-81)

Key Words: angiotensin II receptor antagonist, candesartan cilexetil, CV-11974, TCV-116, enalapril

The advantages of using angiotensin converting enzyme (ACE) inhibitors as antihypertensive agents are as follows: 1) they have a mild antihypertensive action without producing reactive tachycardia; 2) they seldom cause adverse metabolic effects or affect the functions of the brain, heart, and kidney; and 3) there is no rebound phenomenon when the treatment is discontinued. However, ACE inhibitors can induce cough (1, 2) and angioedema (3, 4), both of which are thought to be mediated by increased levels of inflammatory peptides such as bradykinin (BK) and substance P.

Since the discovery of losartan, a clinically effective angiotensin (Ang) II receptor antagonist (5), many nonpeptide Ang II receptor antagonists have been developed for use as antihypertensive agents. Differences in the antihypertensive properties of Ang II antagonists and ACE inhibitors are currently under investigation.

Candesartan cilexetil (TCV-116) is a prodrug of candesartan (CV-11974), an Ang II receptor antagonist with high selectivity for AT1 receptors (6-8). It specifically inhibits Ang II-induced vasoconstriction in the rabbit aorta and potently inhibits the

Ang II-induced pressor response in rats (9). In several models of hypertension in rats, including spontaneously hypertensive rats (SHR) and 2-kidney, 1-clip hypertensive rats (2K, 1C-HR), TCV-116 produced a clear dose-dependent reduction in blood pressure at oral doses of 0.1 to 10 mg/kg. However, it did not lower blood pressure in deoxycorticosterone acetate/salt hypertensive rats (DOCA/salt-HR) (10, 11).

In the present study, the antihypertensive effects of TCV-116 were compared with those of the ACE inhibitor enalapril in SHR, 2K, 1C-HR and 1-kidney, 1-clip hypertensive rats (1K, 1C-HR).

Materials and Methods

Blood Pressure Responses Induced by Vasoactive Substances

Male 8-10-week-old Sprague-Dawley rats, weighing 300-400 g, were purchased from the Japan Clea Laboratory. They were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and their femoral veins and arteries were cannulated with polyethylene tubes. The catheters, filled with

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heparinized saline, were passed s.c. and exteriorized dorsally at the neck. The animals were then placed in plastic cages overnight and allowed to move freely; tap water was given *ad libitum*, but solid food was withheld. The experiments were started the next morning. The mean blood pressure was recorded for each animal on a pen-writing polygraph (San-ei 8662E, Japan) connected to a pressure transducer (San-ei 45277, Japan) via the aortic cannula. The pressor responses to Ang I (300 ng/kg), Ang II (100 ng/kg), Ang III (300 ng/kg), and norepinephrine (NE, 1 μ g/kg), and the depressor response to bradykinin (BK, 3 μ g/kg), all of which were injected separately into the femoral vein, were measured twice, and the average response to each drug was used as the control for all calculations. After the blood pressure had returned to pretreatment levels, TCV-116 and enalapril, suspended in 0.5% (w/v) gum arabic in tap water, were administered orally in a volume of 5 ml/kg. Thereafter, Ang I, Ang II, Ang III, NE, or BK were injected repeatedly at given times, and the inhibition of the pressor response or augmentation of the depressor response was determined.

Antihypertensive Effects of TCV-116 and Enalapril in Spontaneously Hypertensive Rats

Male, 20-21-week-old spontaneously hypertensive rats (SHR: Ta), with mean arterial blood pressure of 150-200 mmHg and weighing 240-350 g, were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The abdominal aorta of each rat was cannulated via the femoral artery, with a polyethylene tube (PE-10 fused to PE-50) filled with heparinized saline. The catheter was passed s.c., exteriorized on the neck, and secured with a harness and spring attached to a water-tight swivel (Instech 375/22, USA). The animals were allowed to recover for 1 day in individual plastic cages. TCV-116 and enalapril were then administered as a suspension with gum arabic in a volume of 2 ml/kg; control rats received 2 ml/kg of vehicle only. The mean blood pressure was recorded for 24 h after the drugs or vehicle were administered, as previously described. The animals were allowed free access to a standard diet (CE-2, Japan Clea Laboratories), and tap water during the experiments.

Antihypertensive Effects of TCV-116 and Enalapril in 2- and 1-Kidney, 1-clip Hypertensive Rats

Male 6-week-old Wistar rats, weighing 150-160 g (Japan Clea Laboratory), were anesthetized with sodium pentobarbital (50 mg/kg i.p.). After making a dorsal incision, the left renal artery was constricted by applying a silver clip (internal diameter, 0.22-0.27 mm); the right kidney was either left intact for 2K, 1C-HR, or removed for 1K, 1C-HR. After surgery, the rats were maintained on a standard diet (CE-2) for 4 to 6 weeks and given tap water *ad libitum*. Animals with a mean arterial blood pressure of 150-230 mmHg were selected, and as 1K, 1C-HR, those with a plasma-renin-concentration (PRC) of < 45 ng Ang I /ml/h were used. Blood pressure was measured as previously de-

scribed. Arterial blood (approximately 200 μ l), was collected from the tail into a sample tube containing 5 μ l of 6% EDTA. The plasma samples were obtained by centrifugation and were stored at -80°C until required. PRC was measured as previously described (12). Briefly, plasma samples (10 μ l) were incubated for 90 min at 37°C with angiotensinogen (90 μ l), 8-hydroxyquinoline and 2,3-dimercaptopropanol. The Ang I generated by the renin in the rat plasma was measured using an RIA kit (CEA-IRA-SORIN, France). The PRC was expressed per ng of Ang I generated by 1 ml of plasma in 1 h. Angiotensinogen was prepared from the plasma of nephrectomized rats by the method of Haas *et al.* (13).

Measurement of Plasma Angiotensin Converting Enzyme Activity in Spontaneously Hypertensive Rats

The plasma ACE activity was measured using a radioenzymatic assay, as described by Rohrbach (14). Plasma (25 μ l) from 20-21-week-old male SHR (SHR: Ta, body weights, 260-320 g) was incubated for 30 min at 37°C with [14 C]hippuryl-L-histidyl-L-leucine ([14 C]HHL) in a total volume of 100 μ l. The reaction was stopped by adding 50 μ l of 1 N HCl, and the [14 C]hippuric acid cleavage product was extracted with 400 μ l of ethyl acetate. Radioactivity in the extract was measured using a liquid scintillation spectrometer (LKB-1216 Rackbeta, USA). The ACE activity was expressed as the rate of production of [14 C]hippuric acid (nmol/min/ml plasma).

Statistical Analysis

All values are expressed as the mean $\pm$ S.E.M. Values for the different groups were compared using one-way analysis of variance (ANOVA) and Dunnett's *t*-test or Student's *t*-test for paired data. Two-tailed *P* values of < 0.05 were regarded to indicate statistical significance.

Drugs

Candesartan cilexetil (TCV-116; ($\pm$)-1-(cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylate), candesartan (CV-11974; 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylic acid), enalapril, and enalaprilat were synthesized by Takeda Chemical Industries. The remaining drugs and agents were purchased as follows: angiotensins (Ang I, Ang II and Ang III) and bradykinin from the Peptide Institute, Inc., Japan, (-)-norepinephrine ((-)-Arterenol Bitartrate[®]) and hippuric acid from Sigma Chemical Co., USA, [14 C]hippuryl-L-histidyl-L-leucine from DuPont, USA, sodium pentobarbital (Nembutal[®] Injection) from Dynabot, USA, heparin (Heparin Sodium Injection-NR) from Shimizu Pharmaceutical Co., Japan, disodium ethylenediamine tetra-acetate dihydrate from Dojin Chem. Co., Japan, and 8-hydroxyquinoline and 2,3-dimercaptopropanol from CEA-IRE-SORIN, France.

Fig. 1. Effects of TCV-116 and enalapril on pressor responses to angiotensin I (AI, 300 ng/kg i.v.), angiotensin II (AII, 100 ng/kg i.v.), angiotensin III (AIII, 300 ng/kg i.v.) and norepinephrine (NE, 1 µg/kg i.v.) and the depressor response to bradykinin (BK, 3 µg/kg i.v.) in the normotensive rat. Blood pressure responses to vasoactive substances were measured just before (open columns) and 1 h after oral administration of TCV-116 (1 mg/kg, shadowed columns) and enalapril (3 mg/kg, hatched columns). Values represent the mean $\pm$ S.E.M. of changes in blood pressure. Five rats were used in each group.

Results

Effects of CV-11974 and Enalaprilat on Plasma Angiotensin Converting Enzyme Activity in Spontaneously Hypertensive Rats

Enalaprilat, at a concentration of 10^{-7} M, almost completely inhibited plasma ACE activity (55.9 ± 3.6 to 1.4 ± 0.6 nmol/min/ml, 97.5% inhibition, $n = 3$). In contrast, CV-11974 had no effect on the ACE activity, even at a concentration of 10^{-5} M (55.9 ± 3.6 to 53.7 ± 3.9 nmol/min/ml, 4.2% inhibition, $n = 3$).

Effects of TCV-116 and Enalapril on Blood Pressure Responses Modulated by Vasoactive Substances

Blood pressure responses to vasoactive substances were examined just before and 1 h after oral administration of TCV-116 and enalapril to normotensive rats (Fig. 1). TCV-116, at a dose of 1 mg/kg, completely inhibited the pressor responses induced by Ang I, II, and III, but it had little or no effect on the pressor and depressor responses induced by NE and BK, respectively. Enalapril, at a dose of 3 mg/kg, completely inhibited the Ang I-induced pressor response and markedly potentiated the BK-induced depressor response, but it had little or no effect on the pressor responses induced by Ang II, Ang III and NE.

Antihypertensive Action of TCV-116, CV-11974, and Enalapril in Spontaneously Hypertensive Rats

Just before drug administration, the mean blood pressure for all SHR in this study was 166.5 ± 1.5 mmHg ($n = 70$). Enalapril, at a dose of 3 mg/kg p.o., reduced blood pressure by 30 mmHg 2–5 h after administration. This effect was maximal, since doses of 10 and 30 mg/kg enalapril caused no further reduction, although the antihypertensive effect persisted somewhat longer at these higher doses (Fig. 2a). These findings suggest that a single dose of 3 mg/kg is sufficient to study the maximum anti-

hypertensive effect of enalapril. At a dose of 10 mg/kg p.o., TCV-116 lowered blood pressure by 45–50 mmHg 3 to 7 h after administration (Fig. 2b). Concomitant administration of 10 mg/kg enalapril produced a similar antihypertensive effect to that of TCV-116 alone.

Oral administration of 10 mg/kg enalapril, followed 3 h later by 0.1 mg/kg CV-11974 i.v., produced a reduction in blood pressure greater than that caused by enalapril alone (Fig. 2c). The reduction was similar to that produced by the concomitant oral administration of TCV-116 and enalapril. However, additional i.v. administration of enalaprilat at a dose that completely inhibits the Ang I-induced pressor response (0.3 mg/kg) (15) failed to reduce the blood pressure further.

Antihypertensive Action of TCV-116 and Enalapril in 2- and 1-Kidney, 1-clip Hypertensive Rats

At the time of drug administration, the mean blood pressure for all 2K, 1C-HRs in this study, was 191.3 ± 3.5 mmHg ($n = 42$). In this rat model, TCV-116, at doses of 0.1 to 10 mg/kg p.o., lowered blood pressure in a dose-related manner. Ten mg/kg TCV-116 lowered blood pressure by a maximum of 70 mmHg, and this antihypertensive effect lasted for more than 24 h. Enalapril, at doses of 1 and 10 mg/kg p.o., also dose-dependently lowered blood pressure. However, its maximum antihypertensive effect was about 50 mmHg (Fig. 3a).

The mean blood pressure for all 1K, 1C-HRs in this study was 188.7 ± 3.1 mmHg ($n = 25$) at the time of drug administration. In this group, TCV-116, at doses of 0.1, 1, and 10 mg/kg p.o., dose-dependently lowered blood pressure. Ten mg/kg of TCV-116 lowered blood pressure by a maximum of about 50 mmHg and this antihypertensive effect lasted more than 24 h. However, in 1K, 1C-HRs, enalapril, even at a dose of 10 mg/kg p.o., did not cause any significant reduction in blood pressure (Fig. 3b).

Discussion

Unlike enalaprilat, the use of HHL as an artificial substrate of ACE enabled us to demonstrate that CV-11974, which is the biologically active form of TCV-116, has no activity as an ACE inhibitor. In normotensive rats, orally administered TCV-116 did not have any effect on the NE-induced pressor response or the BK-induced depressor response, but it specifically inhibited the pressor responses induced by angiotensins (Ang I, Ang II, and Ang III). Ang I is converted to Ang II by intrinsic ACE, and Ang II is further metabolized to Ang III, which also induces a pressor response via Ang II receptors (16). These findings indicate that orally administered TCV-116 specifically inhibits Ang II receptors, with no inhibitory activity for ACE. On the other hand, enalapril reduced the Ang I-induced pressor response, and potentiated the BK-induced depressor response. Both of these properties are characteristic of an ACE inhibitor, since ACE is the same enzyme as kininase II (17), which not only converts Ang I to Ang II, but also degrades BK.

Although the Ang II receptor antagonist and the ACE inhibitor both act on the renin-angiotensin system, differences in their inhibitory mechanisms most probably account for their different antihypertensive effects.

In SHR, enalapril at 3 mg/kg p.o. reduced blood pressure by approximately 30 mmHg, and this effect was maximal, since higher doses caused no further reduction. TCV-116 showed dose-dependent antihypertensive effects at doses of 0.01, 0.1, 1, and 10 mg/kg p.o. in a previous study (11). In the present study, 10 mg/kg TCV-116 reduced blood pressure by 45–50 mmHg. Although the antihypertensive effect of more than 10 mg/kg of TCV-116 has not been investigated yet, a comparable or greater effect can be expected at higher doses.

The maximum antihypertensive effect of TCV-116 at 10 mg/kg was greater than that of enalapril at doses up to 30 mg/kg. Furthermore, concomitant administration of both drugs produced a similar antihypertensive effect to that of TCV-116 alone. The maximum antihypertensive effect of enalapril was increased by the i.v. administration of CV-11974, whilst enalaprilat had no additional effect. Enalapril plus the i.v. administration of EXP3174, an active losartan metabolite, lowered blood pressure to the same degree as enalapril plus CV-11974 (data not shown). The maximum antihypertensive effects produced by the Ang II receptor antagonists were similar, irrespective of their routes of administration. Since enalapril significantly potentiated the BK-induced depressor response, this effect could have contributed to its antihypertensive action in SHR, particularly if intrinsic BK had acted spontaneously to prevent hypertension in these animals. However, this potentiation of the BK-response is unlikely to be important, since enalapril plus TCV-116 had no additional antihypertensive effect as compared with TCV-116 alone. In rats with renal hypertension, the antihypertensive effect of capto-

Fig. 2. Comparison of the antihypertensive activity of TCV-116 or CV-11974 and enalapril or enalaprilat in spontaneously hypertensive rats. *a)* The maximum decrease in blood pressure caused by enalapril at doses of 3 (●), 10 (△), and 30 (■) mg/kg were all similar at about 30 mmHg. *b)* TCV-116 and enalapril were administered concomitantly. Vehicle ○, TCV-116 10 mg/kg p.o. ●, enalapril 10 mg/kg p.o. ▲, TCV-116 10 mg/kg p.o., and enalapril 10 mg/kg p.o. ■. *n* = 6–10. **p* < 0.05, vs. control or the enalapril-treated group (ANOVA). *c)* Enalapril maximally reduced blood pressure 3 h after its administration. At this time, CV-11974 or enalaprilat was also given. C: control, E: enalapril 10 mg/kg p.o. *n* = 3–5. **p* < 0.05, vs. the value before (control) or 3 h after enalapril treatment (Student's *t*-test for paired data).

pril was intensified by combination with the non-peptide Ang II receptor antagonist EXP6803 (18) or losartan (19). Since the effects of neither EXP6803 nor losartan were increased by combination with

Fig. 3. Effects of oral administration of TCV-116 and enalapril on blood pressure in 2-kidney, 1-clip hypertensive rats (2K, 1C-HR) (a) and 1-kidney, 1-clip hypertensive rats (1K, 1C-HR) (b). Values are expressed as the mean $\pm$ S.E.M. of changes in blood pressure. Five to 7 rats were used in each group. * $p < 0.05$, vs. controls (Dunnett's t-test).

Fig. 4. Comparison of the maximum antihypertensive effects of TCV-116 (T) and enalapril (E) in spontaneously hypertensive rats (SHR), 2-kidney, 1-clip hypertensive rats (2K, 1C-HR) and 1-kidney, 1-clip hypertensive rats (1K, 1C-HR). Values are expressed as the mean $\pm$ S.E.M. of changes in blood pressure. Five to 10 rats were used in each group. * $p < 0.05$, vs. the enalapril-treated group (Dunnett's t-test).

captopril (16, 17), the potentiation of BK, prostaglandins, or both, by ACE inhibitors (20, 21) may contribute little to their antihypertensive effect.

The maximum antihypertensive effects of TCV-116 and enalapril were compared in three models of hypertensive rats (Fig. 4). Although both of these drugs dose-dependently reduced blood pressure in SHR and 2K, 1C-HR, the maximal antihypertensive effect of TCV-116 was more marked than that of enalapril in these models.

Enalapril did not have any significant anti-hypertensive action in 1K, 1C-HR, but 0.1 to 10 mg/kg TCV-116 p.o. dose-dependently reduced the blood pressure in these animals. Losartan at a dose of 100 mg/kg p.o. also lowered the blood pressure in 1K, 1C-HR (data not shown). In 1K, 1C-HR, the renin-angiotensin system is activated only in the early stage, but is considered not to be involved in the subsequent maintenance of hypertension (22, 23). In fact, in the present study, the PRC was at the normal level of < 45 ng Ang I/ml/h in 1K, 1C-HR. In addition, the plasma ACE activity and aortic renin concentration in 1K, 1C-HR, did not differ from those in normotensive Wistar rats (12). Ang II is probably involved in the maintenance of hypertension in 1K, 1C-HR, since Ang II receptor antagonists significantly lowered the blood pressure in these animals, whilst enalapril had little or no effect. These findings suggest that the renin-angiotensin system may be inhibited to a greater extent by TCV-116 than by enalapril.

There are two possible explanations why TCV-116, but not enalapril, inhibited the renin-angiotensin system in SHR, 2K, 1C-HR, and 1K, 1C-HR: 1) there is another system besides renin and ACE that is capable of generating Ang II, and 2) ACE is inaccessible to enalapril. These possibilities should be investigated further. However, alternative Ang II-generating pathways, besides renin and ACE, have recently been found (24-28). For example, in 1K, 1C-HR, the pressor response to renin substrate was not inhibited by the ACE inhibitor captopril, but it was inhibited by the Ang II receptor antagonist

saralasin (29). Chymase does not generate Ang II in rats (30), but the existence of enzymes such as cathepsin G (31), tonin (32, 33), kallikrein (34), and cytotoxic cell protease (granzyme) (35), all of which can generate Ang II, may help to explain the different antihypertensive effects caused by TCV-116 and enalapril. TCV-116 was more potent than enalapril at lowering blood pressure in all three animal models, and the magnitude of the difference increased in the order of 2K, 1C-HR, SHR and 1K, 1C-HR. Although the PRC was within the normal range in SHR and 1K, 1C-HR, tissue Ang II generation is activated. In addition, the alternative Ang II generating enzymes mentioned above may be activated to a greater extent in 1K, 1C-HR, than in the other two models.

These findings suggest that Ang II receptor antagonists may become the most effective drugs to inhibit the action of Ang II. Differences in the magnitude of the antihypertensive effect of the two drugs in the three hypertensive rat models may reflect differences in their efficacy in clinical studies. In order to prove that the antihypertensive action of Ang II receptor antagonists is superior to that of ACE inhibitors, it is necessary to study a range of ACE inhibitors with higher affinity to tissue than enalapril. Furthermore, TCV-116 may pass across the blood-brain barrier, and block Ang II receptors in the brain, as suggested by Kohara *et al.* (36). Therefore, maximum antihypertensive effects of intracerebroventricularly administered CV-11974 should also be compared with those of enalaprilat in these animal models.

The present findings indicate that TCV-116 is more potent than enalapril at reducing blood pressure in SHR, 2K, 1C-HR, and 1K, 1C-HR. This may be because some of the ACE escapes inhibition.

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