Effects of the Non-Peptide Angiotensin II Receptor Antagonist TCV-116 on Systemic and Renal Hemodynamics in Dogs with Renal Hypertension

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The effects of TCV-116, a new non-peptide angiotensin II receptor antagonist, on systemic and renal hemodynamics were studied in conscious normotensive and renal hypertensive (2-kidney, 1-clip Goldblatt type) dogs. When orally administered at 0.03 to 1.0 mg/kg, TCV-116 inhibited the pressor response to angiotensin II in conscious normotensive dogs in a dose-dependent fashion. The IC_{50} and IC_{100} values were 0.06 mg/kg and 0.86 mg/kg, respectively. TCV-116 at doses of 0.3 mg/kg and 1.0 mg/kg dose-dependently and persistently decreased systolic and diastolic blood pressure in both dogs with acute renal (hyperreninemic) and those with chronic renal (normoreninemic) hypertension. Even a high dose of TCV-116 (10 mg/kg, p.o.) increased effective renal plasma flow without affecting blood pressure or glomerular filtration rate in normotensive dogs. Furthermore, even at this high dose, TCV-116 did not reduce effective renal plasma flow or glomerular filtration rate in dogs with renal hypertension despite marked reduction in systemic blood pressure. The angiotensin converting enzyme inhibitor enalapril (10 mg/kg, p.o.) had renal hemodynamic effects similar to those of TCV-116. These findings indicate that TCV-116 has potent hypotensive effects not only in dogs with acute renal hypertension but also in those with chronic renal hypertension, but does not appear to adversely affect renal hemodynamics. (*Hypertens Res* 1995; 18: 69-75)

Key Words: non-peptide angiotensin receptor antagonist, TCV-116, renal hypertensive dogs, renal hemodynamics

The renin-angiotensin system has long been known to play an important role in the pathophysiology of several circulatory disorders, and the use of angiotensin converting enzyme (ACE) inhibitors for the pharmacological blockade of this system in the treatment of hypertension and congestive heart failure has clearly proven beneficial (1). The great clinical success of ACE inhibitors has generated considerable interest in the development of specific pharmacological blockers of the renin-angiotensin system, and in particular, angiotensin II (AII) receptor antagonists. Recently, Kubo and co-workers (2) synthesized TCV-116, a highly potent AII receptor antagonist. *In vitro* studies by Shibouta *et al.* (3) have demonstrated that CV-11974, the active metabolite of TCV-116, is a potent non-peptide antagonist with high affinity, specificity and selectivity for the AII receptor (Type AT_1). Furthermore, TCV-116 has been shown to decrease blood pressure in rats with various types of experimentally induced hypertension (4).

In the present study, we first examined the inhibitory effect of TCV-116 on the pressor response to AII in conscious normotensive dogs. We then ex-

amined the antihypertensive effect of TCV-116 in conscious dogs with acute or chronic renal hypertension, and finally evaluated the effect on renal hemodynamics of a overdose of TCV-116 in conscious dogs with renal hypertension.

Methods

1. Inhibitory Effect of TCV-116 on Pressor Response to AII

Male beagle dogs weighing 10 to 13 kg were prepared for blood pressure recording as follows. They were first anesthetized with sodium pentobarbital (30 mg/kg, i.v.). One catheter was then positioned in the lower abdominal aorta via the femoral artery for measurement of systemic blood pressure, and another was positioned in the inferior *vena cava* via the femoral vein for AII administration. Each catheter was led through a subcutaneous tunnel on the animal's back and secured to the skin between the scapulae. The dogs were permitted an approximately 5-day period for recovery from surgery. After establishing a basal pressor response to AII (0.1 μ g/kg, i.v.), TCV-116 was administered orally.

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Thereafter, AII was injected repeatedly at 1, 2, 5, 7, 10 and 24 h after TCV-116 treatment. Pressor responses to AII before and after the administration of TCV-116 were determined, and the inhibitory effect of drugs on the pressor response was evaluated as percent change in pressor response. The IC_{50} and IC_{100} values were calculated from the area over the time-effect curve from 0 to 10 h.

2. Antihypertensive Effect

Male beagle dogs weighing 12 to 13 kg were used. They were first anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The right renal artery was then exposed through a flank incision and was constricted with a silver clip to reduce renal arterial blood flow to 40% of the initial value. Dogs were tested 3 days after surgery as models of acute phase (hyperreninemic) hypertension. More than 8 weeks after initiation of renal arterial constriction, those dogs with a mean blood pressure of 140 mmHg or higher while conscious were used as models of chronic phase (normoreninemic) hypertension. Dogs were trained to sit quietly in their cage. Blood pressure and heart rate were measured indirectly with an electro-sphygmomanometer (Narco, RE-300) consisting of an inelastic cuff containing an inflatable rubber bag. The sphygmomanometer cuff was applied to the foreleg. Measurements were made 0.5, 1, 2, 3, 5, 7, 10 and 24 h after oral administration of drugs.

Plasma renin activity was measured by radioimmunoassay using a commercially available kit (Renin Riabead, Dinabot).

3. Renal Hemodynamic Effect of TCV-116 and Enalapril

Normotensive and chronic renal hypertensive dogs were used. The effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were measured by the single-injection technique using para-aminohippuric acid (PAH) and iohalamate by the method of Fujiwara *et al.* (5). In brief, a mixture of PAH and iohalamate (30 mg/kg) was injected intravenously, and blood samples were obtained 0, 5, 10, 20, 30, 60, 90, 120 and 180 min later. The plasma concentrations of PAH and iohalamate were determined by reversed-phase high performance liquid chromatography (column: YMC-Pack A-312, HPLC system: Hitachi Model L-6200). In brief, 100 μ l of plasma was mixed with 5% perchloric acid and centrifuged. The supernatant was neutralized with 1N NaOH, and a 50 μ l aliquot was subjected to HPLC. The sample was eluted with 0.1 M NaH_2PO_4 /methanol (85:15) at a flow rate of 1.0 ml/min, and the optical density was read at a wavelength of 245 nm. The retention times of PAH and iohalamate were approximately 5 and 8 min, respectively. ERPF and GFR were calculated as follows:

$$ERPF = Q_{PAH}/AUC_{PAH}$$

$$GFR = Q_{IOT}/AUC_{IOT}$$

where Q_{PAH} and Q_{IOT} were the total amounts of PAH and iohalamate injected, and AUC_{PAH} and AUC_{IOT} were the areas under the plasma

concentration-time curves of these indicators from 0 to 180 min.

After measurement of blood pressure, ERPF and GFR, TCV-116 or enalapril was administered orally, and blood pressure, ERPF and GFR were measured 2 and 24 h later.

All values were expressed as means $\pm$ SEM. Bonferroni's test for paired values was used to compare measurements before and after treatment. These analyses were performed using the SAS GLM procedure (6). *p* Values below 0.05 were considered to indicate statistical significance.

Results

1. Inhibitory Effect of TCV-116 on Pressor Response to AII

The basal pressor responses to AII in groups treated with 0.003, 0.03, 0.3 and 1.0 mg/kg of TCV-116 were 48.3 ± 3.7 , 44.2 ± 4.0 , 47.3 ± 4.4 and 37.8 ± 3.5 mmHg, respectively. There was no difference among these groups. TCV-116 attenuated the pressor response to AII in a dose-dependent fashion (Fig. 1). Maximum inhibition at 0.03 mg/kg of TCV-116 was observed 3 to 5 h after administration. At this dose, inhibition rates were $40 \pm 5.0\%$ at 3 h and 50 ± 9.8 at 5 h. At 0.3 mg/kg, the rate of inhibition was $88.7 \pm 2.9\%$ at 3 h, and the inhibitory effect lasted for up to 10 h. At 1.0 mg/kg, the pressor response to AII was completely eliminated for 10 h. The calculated IC_{50} and IC_{100} values were 0.06 mg/kg and 0.86 mg/kg, respectively (Fig. 2).

TCV-116 at 1 mg/kg did not affect the pressor response to norepinephrine or the depressor response to bradykinin (data not shown).

Fig. 1. Effect of TCV-116 on the pressor response to AII. TCV-116 was given orally to conscious beagle dogs, and the change in the pressor response to AII (0.1 μ g/kg, i.v.) was observed. Each point represents the mean $\pm$ SEM. Significant differences between the baseline value and experimental period: * $p < 0.05$; ** $p < 0.01$. Details are described in the text.

2. Antihypertensive Effect of TCV-116 in Dogs with Renal Hypertension

Acute phase of hypertension: As shown in Fig. 3, dogs tested 3 days after surgery had high systolic (203.1 ± 2.5 mmHg) and diastolic (109.1 ± 0.9 mmHg) blood pressures and increased PRA of approximately 5 ngAI/ml/h. Basal heart rate was 87.2 ± 5.5 beats/min. There were no differences in basal blood pressure or heart rate among the 0.1, 0.3 and 1.0 mg/kg TCV-116 groups.

TCV-116 reduced both systolic and diastolic blood pressures in a dose-dependent fashion at 0.3 to 1.0 mg/kg (Fig. 4). TCV-116 administered orally at 0.3 mg/kg reduced systolic and diastolic blood pressures by 27 and 29 mmHg, respectively; these effects lasted for 7 h after administration. TCV-116 administered orally at 1 mg/kg markedly reduced both systolic and diastolic blood pressures by about 45 mmHg, and its hypotensive effect lasted for 10 h. Heart rate was not affected by TCV-116.

Chronic phase of hypertension: As shown in Fig. 3, dogs tested 1 to 2 months after surgery had high systolic (207.8 ± 3.8 mmHg) and diastolic (115 ± 1.6 mmHg) blood pressures, but PRA had normalized to 1 to 2 ngAI/ml/h. There were no differences in basal blood pressure or heart rate among the 0.1, 0.3 and 1.0 mg/kg TCV-116 groups.

TCV-116 at doses of 0.3 to 1.0 mg/kg reduced blood pressure in a dose-dependent fashion (Fig. 5). TCV-116 administered orally at 0.3 mg/kg reduced both systolic and diastolic blood pressures by about 30 mmHg, and its hypotensive effect lasted for 7 h. TCV-116 administered orally at 1 mg/kg reduced both systolic and diastolic blood pressures by about 50 mmHg, and its hypotensive effect lasted for 10 h. Heart rate was not affected by TCV-116.

Fig. 2. The relationship between the area (the area over the inhibitory effect-time curve shown in Fig. 1) and dose given to the dogs. The IC_{50} and IC_{100} values of TCV-116 on the pressor response to AII ($0.1 \mu\text{g/kg}$, i.v.) were calculated.

Fig. 3. Changes in blood pressure and plasma renin activity after renal arterial stenosis in beagle dogs. SBP, systolic blood pressure; DBP, diastolic blood pressure; PRA, plasma renin activity. Significant differences between the baseline value and experimental period: * $p < 0.05$; ** $p < 0.01$. Details are described in the text.

Fig. 4. The hypotensive effect of TCV-116 on systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in acute 2-kidney, 1-clip renal hypertensive dogs. TCV-116 was given orally. Each point represents the mean $\pm$ SEM of 4 dogs. Significant differences between the baseline value and experimental period: * $p < 0.05$; ** $p < 0.01$. Details are described in the text.

3. Effects of TCV-116 and Enalapril on Renal Hemodynamics

Basal blood pressure, ERPF and GFR in normotensive dogs in this study were similar in the control, TCV-116 and enalapril groups. TCV-116 administered orally at 10 mg/kg had no effect on mean blood pressure, but significantly increased ERPF. It had no effect on GFR (Fig.6). Enalapril at 10 mg/kg also had no effect on mean blood pressure or GFR, but significantly increased ERPF.

In dogs with chronic renal hypertension, the basal mean blood pressure, ERPF and GFR were similar in all three treatment groups. TCV-116 administered orally at 10 mg/kg reduced mean blood pressure 3 to 5 h later by about 45 mmHg, but had no effect on ERPF or GFR (Fig.7). Enalapril administered orally at 10 mg/kg reduced mean blood pressure to the same extent as TCV-116 did, and also had no effect on ERPF or GFR (Fig.7).

Discussion

Our findings show that TCV-116, a non-peptide AII receptor antagonist, suppresses in a dose-dependent fashion the pressor response to AII in conscious normotensive dogs and reduces in a dose-dependent fashion blood pressure not only in conscious dogs with acute renal hypertension but also in those with chronic renal hypertension. TCV-116 had no effect on renal plasma flow or GFR even at high doses in

dogs with renal hypertension.

A dose of 0.3 mg/kg TCV-116 maximally inhibited pressor responses to AII, and continued to have an hypertensive effect for 10 h. Calculated IC_{50} and IC_{100} values were 0.06 and 0.86 mg/kg, respectively. The plasma concentration of CV-11974, the active metabolite of TCV-116, was measured after oral administration of 0.06 mg/kg (IC_{50} value) TCV-116. The time-concentration profile is shown in Fig.8. Even 8 h after administration, the concentration of CV-11974 in plasma was approximately 0.3 ng/ml (0.68×10^{-9} M). These findings clearly show that the inhibitory effect of TCV-116 on the pressor responses to AII is due to blockade of vascular AII receptors. Furthermore, TCV-116 at 1 mg/kg, which completely inhibited the pressor response to AII, did not affect the response to nor-epinephrine or the depressor response to bradykinin. These findings indicate that TCV-116 had a potent and selective inhibitory effect on the vascular AII receptor in the dogs tested in this study.

Fig. 5. The hypotensive effect of TCV-116 on systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in chronic 2-kidney, 1-clip renal hypertensive dogs. TCV-116 was given orally. Each point represents the mean $\pm$ SEM of 4 dogs. Significant differences between the baseline value and experimental period: * $p < 0.05$; ** $p < 0.01$. Details are described in the text.

Fig. 6. Renal hemodynamic effects of TCV-116 and enalapril on mean blood pressure (MBP), effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) in normotensive dogs. TCV-116 and enalapril were given orally at a dose of 10 mg/kg. Each point represents the mean $\pm$ SEM of 4 dogs. Significant differences between the baseline value and experimental period: * $p < 0.05$. Details are described in the text.

Fig. 7. Renal hemodynamic effects of TCV-116 and enalapril on mean blood pressure (MBP), effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) in chronic renal hypertensive dogs. TCV-116 and enalapril were given orally at a dose of 10 mg/kg. Each point represents the mean $\pm$ SEM of 4 dogs. Significant differences between the baseline value and experimental period: ** $p < 0.01$. Details are described in the text.

Following these findings, the antihypertensive effect of TCV-116 was evaluated in 2-kidney, 1-clip Goldblatt hypertensive dogs in which the renal artery had been constricted with a silver clip to reduce renal blood flow by about 60%. PRA 2 to 3 days after surgery was significantly elevated and accompanied by an increase in systemic blood pressure. We used these dogs as a model of acute phase (hyperreninemic) hypertension. TCV-116 significantly reduced both systolic and diastolic blood pressure in a dose-dependent fashion. The hypotensive effect at 0.3 mg/kg lasted for 5 h, while at 1 mg/kg it lasted for 10 h. These findings indicate that TCV-116 reduced blood pressure via peripheral vasodilation by antagonizing elevated levels of circulating AII in dogs with acute renal hypertension.

In dogs with chronic renal hypertension, TCV-116 reduced both systolic and diastolic blood pressures, and the blood pressure profile over time was very similar to that observed for the acute renal hypertensive model. In our dogs with chronic renal

Fig. 8. Changes in plasma CV-11974 concentration after oral administration of TCV-116 to dogs. TCV-116 was given at a dose of 0.06 mg/kg (IC_{50} value), and the plasma concentration of CV-11974, the active metabolite of TCV-116, was measured.

hypertension, PRA returned to basal level, even though blood pressure remained high. This is consistent with the findings of previous studies (7, 8). Miyazaki *et al.* (8) reported that increased vascular AII production plays a role in maintaining chronic renal hypertension. The reduction in blood pressure achieved by ACE inhibitors in dogs with chronic renal hypertension is thought to be due to the inhibitory effect of these agents on increased activity of the renin-angiotensin system in vascular tissue (9). AII is known to induce vasoconstriction by direct stimulation of vascular AII receptors. There is evidence for the existence of AII-induced pre-junctional facilitatory effects in peripheral noradrenergic nerve terminals of various tissues including the adrenal gland, heart, blood vessels and kidney (10). In theory, several AII-generating pathways might participate in vascular AII production (11-13). Angiotensin I may be converted to AII by endothelial ACE, extra-endothelial ACE, and other enzymes in the vascular wall, as is the case for the chymostatin-sensitive AII generating enzyme (11). Accordingly, it seems likely that TCV-116 reduced blood pressure by antagonizing local AII produced within the vascular wall in dogs with chronic renal hypertension.

Disturbances of autoregulation of renal blood flow including shifts to the right in the lower limit of the curve have been described for several animal models of hypertension as well as for patients with hypertension (14-19). Furthermore, the degree of disturbance of renal autoregulation has been shown to be related to the severity of hypertension (14). It is therefore important that the renal effects of anti-hypertensive agents be evaluated. We examined the effects on renal hemodynamics of 10 mg/kg of

TCV-116, which was considered to be an overdose, in normotensive dogs and in dogs with renal hypertension. The single injection technique using PAH and iohalamate was employed for measurement of ERPF and GFR. This method has been used for measurement of renal hemodynamics because its results correlate well with those of the standard clearance method (5, 20). TCV-116 at a dose of 10 mg/kg significantly increased ERPF, but had no effect on systemic blood pressure or GFR in normotensive dogs. The same dose of TCV-116 had no effect on cardiac output in conscious normotensive dogs as measured by the thermodilution technique (data not shown). Thus, in these dogs TCV-116 induced renal vasodilation without affecting total peripheral resistance. Chan *et al.* (21) reported that intravenous infusion of DuP 753 increased renal blood flow and GFR in anesthetized dogs without affecting blood pressure, consistent with the results of the present study. These findings taken together suggest that TCV-116 has a renovasodilative effect in normotensive dogs.

In dogs with renal hypertension, TCV-116 at 10 mg/kg reduced mean blood pressure by about 50 mmHg; the hypotensive effect of this dose lasted 24 h, but no effect on ERPF or GFR was noted. AII receptor antagonists usually maintain autoregulation of renal blood flow, but not that of GFR (22). We therefore measured the lower limit of autoregulation of renal blood flow in anesthetized dogs. This value was about 75 mmHg in normotensive dogs, consistent with that determined by Abe *et al.* (23). In the dogs with renal hypertension tested in the present study, the lower limit of autoregulation was about 100 mmHg (data not shown). These findings suggest that TCV-116 did not reduce blood pressure (renal perfusion pressure) below the lower limit of autoregulation of renal blood flow in dogs with renal hypertension. Bovee *et al.* (24) similarly found that intravenous administration of the AII receptor antagonist DuP 753 increased renal blood flow and GFR and decreased blood pressure in spontaneously hypertensive dogs. These findings taken together demonstrate the beneficial effects on renal hemodynamics of AII antagonists. TCV-116 maintained renal blood flow and GFR despite a reduction in systemic blood pressure in dogs with renal hypertension. TCV-116 may thus have beneficial effects on renal function and hemodynamics in patients with essential hypertension.

Because blockade of the renin-angiotensin system with ACE inhibitor(s) has been proven to be beneficial in the treatment of hypertension and congestive heart failure, it seems likely that TCV-116, given its beneficial renal hemodynamic effects, will also prove useful for the treatment of these disorders.

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