Université de Lausanne - Faculté de Médecine
Division d'Hypertension du Département de Médecine interne
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I. Value of different clinical and biochemical correlates to assess angiotensin converting enzyme inhibition.

II. Characterization of the angiotensin II receptor antagonist TCV-116 in healthy volunteers.

THESE

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par

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RESUME

Les deux publications de ce travail caractérisent les effets chez l'homme d'un inhibiteur de l'enzyme de conversion de l'angiotensine et d'un antagoniste de l'angiotensine II, ces deux substances permettant de bloquer l'axe rénine-angiotensine à deux niveaux différents.

Dans la première publication de ce travail, différentes méthodes de mesure du degré d'inhibition de l'axe rénine-angiotensine ont été comparées entre elles lors de l'administration du temocapril, un nouvel inhibiteur de l'enzyme de conversion testé pour la première fois chez l'homme. Les taux plasmatiques du métabolite actif, les taux plasmatiques d'angiotensine I et d'angiotensine II, l'activité plasmatique de l'enzyme de conversion de l'angiotensine et l'effet presseur d'injections intraveineuses d'angiotensine I ont été déterminés de manière répétée avant et après l'administration en double aveugle de 4 différentes doses de temocapril ou de placebo. Le rapport de la concentration de l'angiotensine II sur celle de l'angiotensine I s'est avéré le paramètre le mieux correllé à la fois aux concentrations de métabolite actif ($r=-0.85$, $n=148$) et à l'élévation de pression systolique et diastolique produite par l'injection d'angiotensine I ($r=0.76$ et $r=0.79$, respectivement, $n=148$). Le rapport angiotensine II/angiotensine I est en conclusion un paramètre très fiable permettant de déterminer le degré d'inhibition de l'enzyme de conversion.

Le but de la deuxième étude était de caractériser l'effet d'une substance bloquant les récepteurs de l'angiotensine II, le TCV-116, chez des volontaires normotendus. Quatre différentes doses de TCV-116 (1, 2, 4 et 8 mg) ou un placebo ont été administrées en double aveugle pendant 8 jours à 27 volontaires. Le taux de métabolite actif, les concentrations plasmatiques d'angiotensine II, d'aldostérone, de noradrénaline et d'adrénaline, l'activité de la rénine plasmatique, de même que la réponse de la pression systolique, diastolique et du rythme cardiaque à un bolus intraveineux d'angiotensine II, ont été déterminées de façon répétitive ($n= 17$). Le TCV-116 a diminué de façon dose-dépendante la réponse pressive à l'angiotensine II. Six heures après une dose de 4 mg de TCV-116, la réponse pressive à l'angiotensine II s'est abaissée à 40 ± 4 % de la valeur initiale (moyenne ± SEM, $n=6$) le jour 1 et à 35 ± 8 % le jour 8 du traitement. Les résultats démontrent que le TCV-116 bloque de façon prolongée le système rénine-angiotensine à des doses 7 à 9 fois moins élevées que le losartan, seul antagoniste de l'angiotensine II alors étudié chez l'homme. Par ailleurs, le délai entre une concentration plasmatique donnée du métabolite actif et son effet clinique (inhibition de l'augmentation de pression induite par un bolus intra-veineux d'angiotensine II) au site d'action (hystérèse) a été déterminé pour la première fois lors de l'administration d'un antagoniste de l'angiotensine II. Enfin, malgré une élévation très importante des concentrations plasmatiques d'angiotensine II et de l'activité plasmatique de la rénine induite par le bloquage des récepteurs à l'angiotensine II dès la première dose, aucune diminution de l'effet du TCV-116 n'a été constatée au huitième jour de traitement.

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Value of Different Clinical and Biochemical Correlates to Assess Angiotensin Converting Enzyme Inhibition

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Summary: In a double-blind study, we compared the value of different approaches to assess blockade of angiotensin (Ang) II generation in 10 normal volunteers treated with the new Ang-converting enzyme (ACE) inhibitor temocapril. Plasma concentration of the diacid active metabolite of temocapril, plasma Ang I and II levels, plasma ACE activity, and inhibition of the pressor response to repeated intravenous (i.v.) doses of Ang I were measured before and repeatedly after different doses of temocapril or placebo. In vivo ACE activity, estimated by the plasma Ang II/Ang I ratio, correlated well with temocapril diacid concentration ($r = 0.85$, n = 148) and with systolic and diastolic blood pressure (SBP, DBP) responses to Ang I ($r = 0.76$ and $r = 0.79$, n = 148). SBP and DBP responses to Ang I were also strongly related to temocapril diacid concentration ($r = -0.81$ and $r = -0.88$, n = 148). ACE activity measured in vivo reliably predicts the decrease in Ang-dependent BP to be achieved by ACE inhibitors. **Key Words:** Temocapril—Angiotensin converting enzyme inhibition—Angiotensin II/Angiotensin I ratio—Dose–response relationship—Blood pressure.

Angiotensin (Ang)-converting enzyme (ACE) inhibitors are now widely used in treatment of patients with hypertensive disorders (1–7) and with congestive heart failure (CHF) (8–11). Because of their efficacy, interest in these therapeutic agents has grown tremendously in recent years; several new compounds have been developed, and many are being investigated.

Many years ago, it was demonstrated that the magnitude of blockade achieved by ACE inhibition, the minimum dose needed for maximal efficacy, and onset and duration of action of the agents can be determined with great accuracy by challenging normotensive volunteers with repeat intravenous (i.v.) administrations of Ang I (12). The ability of ACE to cleave various natural and synthetic substrates has been used to measure its activity in vitro. For drug monitoring in patients treated with ACE inhibitors, plasma ACE activity measured in vitro provides consistently reproducible results although absolute ACE activity varies considerably depending on substrates and assay conditions used (13). Finally, because ACE inhibition causes plasma Ang II to decrease and Ang I concentration to increase, an alternative approach to estimating degree of inhibition of ACE activity in vivo is to measure plasma concentrations of Ang I and Ang II and to use the ratio Ang II/Ang I as an estimate of in vivo ACE inhibition (14).

We assessed and compared the accuracy of the three different approaches described to estimate the degree of inhibition of ACE activity, the degree of inhibition of the pressor effect of an i.v. bolus of Ang I, ACE activity in vitro measured with two different substrates, and the Ang II/Ang I ratio (estimating ACE activity in vivo). The new ACE inhibitor temocapril is a prodrug that is converted by deesterification in blood, liver, and intestinal wall (15) to the pharmacologically active metabolite temocapril diacid. After we assessed the pharmaco-

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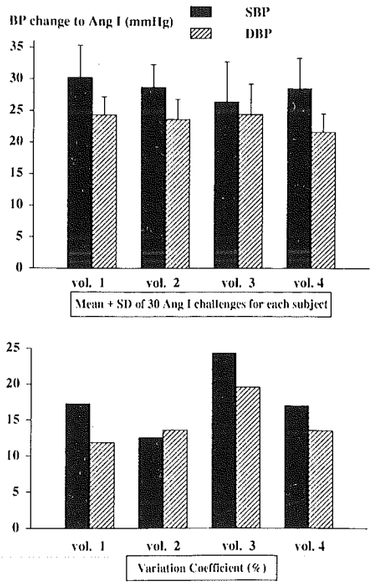

FIG. 2. Maximal systolic blood pressure (SBP) and diastolic BP (DBP) increase (mm Hg, mean + SD) after repeated (n = 30) intravenous bolus of Ang I in the 4 volunteers receiving placebo on 5 different days **(top)** and variation coefficients (%) of maximal pressor effect of Ang I **(bottom).**

teers who entered the study completed it as planned.

BP response to Ang I

Values of BP and HR before and after (''peak value'') Ang I challenge are summarized in Table I. The inhibitory effect of temocapril on both SBP and DBP responses to Ang I was dose dependent (Fig. 1). With all doses, maximum effect was observed within the first 2 h. The lowest dose (2.5 mg) produced an inhibition of the Ang I effect of 47 ± 6% (SBP) and 65 ± 8% (DBP), and the highest doses (20 and 40 mg) produced almost complete inhibition of both SBP and DBP responses. Thus, the minimum effective dose of temocapril is <2.5 mg, and the top of the dose–response curve apparently is reached at ~20 mg with the Ang I dose used in this study. An attenuation of the SBP response to Ang I was still evident 23 h after intake of the 20- and 40-mg doses (inhibition of 15 ± 7 and 28 ± 9%, respectively).

In the control group, administration of placebo had no effect on the pressor response to Ang I. Figure 2 shows the reproducibility of the responses to Ang I injection in the 4 placebo-treated volunteers in a 24-h period on the five separate occasions.

Inhibition of plasma ACE activity in vitro

The time course of ACE activity inhibition measured with the two different substrates is shown in Fig. 3. Major differences were noted in the discriminating power of the two methods of plasma ACE activity measurement. With the substrate Hip-Gly-Gly, ACE activity was reduced almost to zero by all doses of temocapril for ≤4 h after drug administration, whereas duration of the effect on ACE activity was dose dependent. In contrast, inhibitory effects of temocapril on ACE activity measured with the substrate Z-Phe-His-Leu had a distinct dose-dependent relationship at peak effect, ranging from 68 ± 5% (2.5 mg) to 4 ± 0% (40 mg) inhibition in comparison to baseline values. At 24 h postdose, the degree of inhibition of plasma ACE activity was related to the dose of temocapril with both substrates used, ranging from 68 ± 4% (2.5 mg) to 93 ± 1% (40 mg) with Hip-Gly-Gly, and 14 ± 5% (2.5 mg) to 53 ± 6% (40 mg) with Z-Phe-His-Leu.

Plasma Ang I and Ang II

Plasma concentrations of Ang I and Ang II are shown in Table 1. As shown in Fig. 4, plasma Ang I increased and plasma Ang II decreased rapidly and dose dependently after intake of the ACE inhibitor. Plasma Ang II returned toward its baseline level after 23 h with all doses, but plasma Ang I invariably remained increased after active drug.

Inhibition of ACE activity in vivo

The effects of different doses of temocapril and placebo on the time course of plasma Ang II/Ang I ratio are summarized in Table 1 and Fig. 4. This index of in vivo ACE inhibition also showed clear dose dependency at peak effect. At this point, the degree of inhibition ranged from 82 ± 4% (2.5 mg) to 99 ± 0% (40 mg), and at 23 h postdose the degree of inhibition was 44 ± 12 and 80 ± 3% for the 2.5- and the 40-mg doses, respectively.

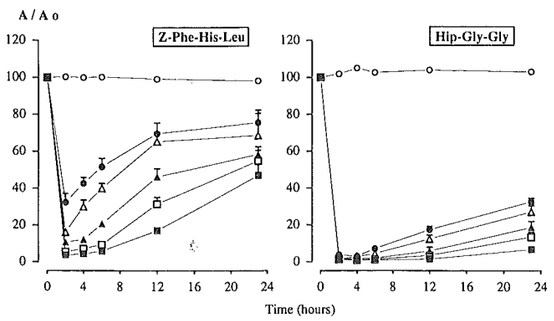

FIG. 3. Time course of plasma angiotensin-converting enzyme (ACE) activity (mean + SEM) of 6 normal volunteers after ingestion of 2.5, 5, 10, 20, and 40 mg temocapril and of 4 normal volunteers after ingestion of placebo on 5 different days (n = 20). **Left:** Z-Phe-His-Leu substrate. **Right:** Hip-Gly-Gly substrate. Results are percentages of pretreatment ACE activity (A/Ao).

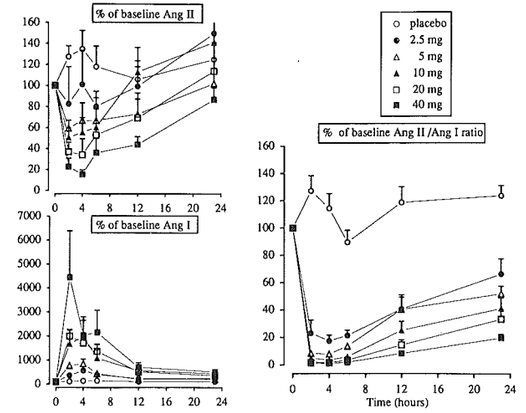

FIG. 4. Time course of plasma angiotensin II (Ang II) **(left, top)** Ang I **(left, bottom)** and Ang II/Ang I ratio **(right)** after 2.5, 5, 10, 20, and 40 mg temocapril (n = 6) or placebo (n = 20) (mean + SEM). Results are percentages of respective pretreatment values.

Temocapril and temocapril diacid plasma concentrations

Plasma concentrations of temocapril and its diacid active metabolite after the five doses of temocapril are shown in Fig. 5. After each dose of temocapril, plasma concentrations of the parent compound increased rapidly, peak values were measured at 1 h (first blood sample), and concentrations subsequently rapidly decreased to almost undetectable levels by ~6 h. The rapid plasma clearance of temocapril was associate with an increase in the concentration of temocapril diacid, formed by the deesterification of temocapril. Peak concentrations were observed 1–2 h after drug administration. Concentrations of temocapril diacid were ~10 times those of the parent drug.

Correlations of effect with plasma temocapril diacid concentrations

SBP and DBP pressor responses to Ang I showed close inverse correlation with the logarithm of the plasma diacid metabolite concentration ($r = -0.81$ and -0.88, $p < 0.001$) (Fig. 6). Similarly, the effects on ACE activity measured in vivo, when expressed as the logarithm, also showed a good inverse correlation with the logarithm of the plasma diacid concentration ($r = -0.85$, $p < 0.001$) (Fig. 6). The correlations between the logarithm of the plasma diacid and the logarithm of ACE activity measured in vitro depended on the method of ACE measurement: It was stronger with the substrate Z-Phe-His-Leu ($r = -0.90$, n = 148, $p < 0.001$) than with the substrate Hip-Gly-Gly ($r = -0.81$, n = 148, $p < 0.001$).

Correlations of effects with ACE activity in vivo and in vitro

The effects on SBP and DBP pressor responses to Ang I showed good correlation with the logarithm of ACE-activity in vivo ($r = 0.76$ and 0.81, respectively, n = 148, $p < 0.001$, (Fig. 7). With ACE-activity measured in vitro, the correlation depended also on the method used: r was 0.83 and 0.67 (Fig. 7) with Z-Phe-His-Leu and Hip-Gly-Gly, respectively.

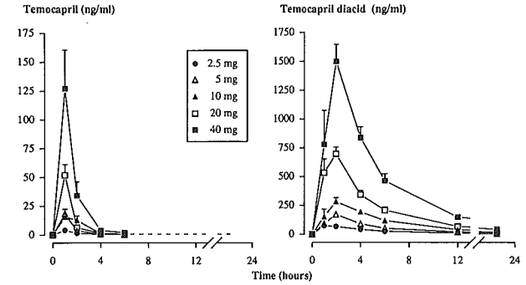

FIG. 5. Plasma temocapril (ng/ml) **(left)** and temocapril diacid (ng/ml) **(right)** concentration after ingestion of temocapril 2.5, 5, 10, 20, and 40 mg (mean + SEM) by healthy volunteers (n = 6). Concentrations of temocapril diacid were ~10-fold those of the parent drug.

DISCUSSION

ACE inhibiting compounds can be characterized with a high degree of accuracy by quantitating their effect on plasma ACE activity and on circulating levels of Ang I and Ang II (23). The Ang II/Ang I ratio is probably the best index by which to assess blockade of ACE activity (14). Recent development of a servo-photoplethysmograph capable of monitoring BP continuously and noninvasively at the finger permits clinical estimation of the effect of an ACE inhibitor on BP response to Ang I. This device has been demonstrated to correlate well with intraarterial pressure (24,25) and to be accurate for study of brief BP changes produced by vasoactive agents in humans (26).

We wished to evaluate the accuracy of these different approaches to characterizing ACE inhibitors and particularly to compare inhibition of the pressor response to Ang I with plasma ACE activity and the circulating levels of the ACE inhibitor. For this pur-

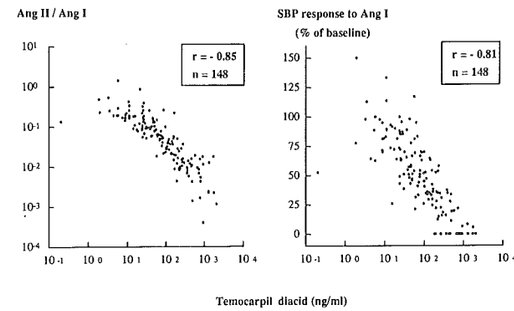

FIG. 6. Correlations between plasma temocapril diacid and angiotensin II (Ang II)/Ang I ratio ($r = -0.85$, n = 148) **(left)** and systolic blood pressure response to Ang I ($r = -0.81$, n = 148) **(right).**

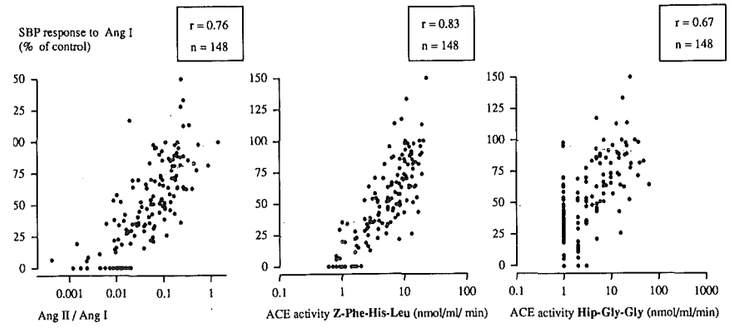

FIG. 7. Correlation between systolic blood pressure (SBP) response to angiotensin I (Ang I) (percentage of basal value) and Ang II/Ang I ratio **(left)** (r = 0.76, n = 148), and in vitro Ang-converting enzyme (ACE) activity determined with the Z-Phe-His-Leu substrate **(center)** (r = 0.83, n = 148) and with the Hip-Gly-Gly substrate **(right)** (r = 0.67, n = 148).

pose, we used a new ACE inhibitor (CS-662, temocapril). Its potent diacid active metabolite was previously shown to have a relatively long duration of action (27,28).

ACE activity measured in plasma provided different information depending on the method and substrate used. The Hip-Gly-Gly method showed long-lasting and intense inhibition of plasma ACE activity and did not detect any major differences between the five different doses (2.5–40 mg) at peak effect since almost complete inhibition was obtained (Fig. 3). In contrast, the Z-Phe-His-Leu method distinguished well between each dose at peak effect as well as during the 23 h after drug intake (Fig. 3). These data confirm results of plasma ACE activity measured in vitro provides no definitive information if conventional assays are used. Depending on the substrate used and on the ACE inhibitor tested, these in vitro measurements may not accurately reflect in vivo ACE activity (13) and more sophisticated methods may be needed (29).

In ACE inhibition, plasma Ang II concentration, measured after high-performance liquid chromatography separation (21), appears to be the appropriate endpoint at which to evaluate the degree of renin–angiotensin system blockade (13). Plasma Ang II levels are determined by ACE activity as well as by the precursor decapeptide's concentration (Ang I), which increases significantly after ACE inhibition, which explains why plasma Ang II returned at 23 h toward its initial value even though the pressor effect of Ang I was still inhibited (Figs. 1 and 4). The plasma Ang II/Ang I ratio takes into account the available decapeptide substrate and therefore appears to be the most appropriate index of in vivo ACE activity.

The reproducibility of the SBP response to repeated administration of an Ang I test dose was evaluated in the 4 placebo-treated volunteers, for five 23-h periods, each period separated by a 1-week interval. The coefficient of variation was 18.4 ± 2.6% (mean ± SEM, range 13–24%, (Fig. 2), which corroborates our earlier observations (26). At peak, the 20- and 40-mg doses of temocapril induced almost complete inhibition of the SBP response to Ang I (92 ± 2 and 98 ± 1%, respectively). In addition, with the highest dose, the pressor effect of Ang I of 28 ± 9% was still reduced 23 h after drug intake.

With an ACE inhibitor, we showed for the first time that blockade of the pressor response to Ang I is closely related to the plasma level of the active compound, i.e., the diacid metabolite. In addition, the pressor response to Ang I also correlated strongly with ACE activity in vivo, the parameter we consider to reflect most accurately ACE inhibition (14,30). Consequently, if inhibition of plasma ACE activity is measured accurately, it predicts very well the reduction in Ang II-dependent BP that will be induced by an ACE inhibitor. The correlation between plasma diacid levels and plasma ACE activity or the pressor response to Ang I was analyzed based on a log-linear relation. Indeed, these parameters are expected to exhibit a relation best described by a sigmoidal E_{max} curve. However, since we avoided high and low extremes of drug concentrations in this study, only the linear part of such a curve was explored and linear regression was calculated.

Temocapril is a potent and well-tolerated new ACE inhibitor. With this drug, ACE activity measured in vivo by the plasma Ang II/Ang I ratio was shown to be an excellent predictor of the drug-induced effect on Ang-dependent BP.

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Characterization of the Angiotensin II Receptor Antagonist TCV-116 in Healthy Volunteers

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Abstract The purpose of this study was to assess the inhibitory effect of TCV-116, an orally active angiotensin II (Ang II) antagonist, on the pressor action of exogenous Ang II and to determine the compensatory rise in plasma renin activity and Ang II levels. Twenty-three male volunteers were treated for 8 days in a double-blind fashion with either placebo or TCV-116 (1, 2, or 4 mg PO daily) and challenged on the first, fourth, and eighth days with repeated bolus injections of Ang II. An additional 4 subjects received 8 mg PO daily in a single-blind fashion. The inhibitory effect on the systolic blood pressure response to Ang II was long lasting and clearly dose related. Six hours after 4 mg TCV-116, the systolic blood pressure response to a given dose of Ang II was reduced to 40±4% and 35±8% of baseline value on days 1 and 8, respectively. TCV-116 induced a dose-related increase in plasma renin activity and Ang II levels that was more pronounced on the eighth than on the first day of drug administration. Despite this compensatory mechanism, the relation between the time-integrated systolic blood pressure response to Ang II and the time-integrated CV-11974 levels, the active metabolite of TCV-116, was not different between days 1 and 8. In conclusion, TCV-116 appears to be a well-tolerated, orally active, potent, and long-lasting antagonist of Ang II in men. (***Hypertension*****. 1995;25:14-21.)**

Key Words • angiotensin II • aldosterone • renin • dose-response relationship, drug

Blockade of the renin-angiotensin system has turned out to be a very effective treatment of hypertension and congestive heart failure.[1-4] Today, angiotensin-converting enzyme (ACE) inhibitors are used as therapeutic agents, but originally the concept was established with angiotensin receptor antagonists. Saralasin ([Sar[1],Ala[8]]angiotensin II)[5] was the first receptor antagonist of angiotensin II (Ang II) administered to humans.[6-8] Since this peptide is not orally active, long-term antihypertensive treatment was not possible. Furthermore, the antagonist exhibited significant inherent agonist activity.[9] Therefore, most patients did not respond with a decrease in blood pressure. Furukawa and coworkers[10] have synthesized some imidazole derivatives that specifically block the Ang II–induced vasoconstriction. Important chemical modification of these initial molecules has led to the synthesis of new orally active Ang II receptor antagonists.[11,12] Although most of the compounds specifically block the angiotensin type 1 (AT_1) receptor responsible for all hitherto known Ang II actions, including vascular smooth muscle contraction,[13,14] some compounds that specifically bind to the AT_2 receptors were also synthesized.[13,15-17] The first orally active AT_1 receptor antagonist, DuP 753 (losartan), was shown to effectively block the pressor effect of exogenous Ang II[18,19] and to reduce blood pressure of patients in a manner similar to ACE inhibitors.[20,21] Although losartan is a potent Ang II receptor antagonist, its therapeutic effect is probably mostly due to its active metabolite, EXP3174, which exhibits about 10-fold higher affinity to the receptor.[22]

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TCV-116, a new AT_1 receptor antagonist, and its active metabolite (CV-11974) have been shown in different pharmacological studies to be approximately 10-fold more potent than losartan and to have a long elimination half-life. The objectives of the present study were to assess the inhibitory effect of TCV-116 on the pressor action of exogenous Ang II in healthy volunteers, to determine the dose dependency and duration of the inhibitory effect, to evaluate the correlation of the inhibitory effect with serum levels of the active metabolite CV-11974, and to determine the effect of the compound on plasma Ang II, aldosterone, and catecholamine levels.

Methods

Subjects

Twenty-seven male volunteers aged 20 to 36 years (mean, 26.0 years) and weighing between 48 and 85 kg (mean, 69.9 kg) were enrolled in the randomized, double-blind, placebo-controlled parallel design dose-ranging study. Volunteers underwent a complete physical evaluation, electrocardiogram, and routine blood and urine analyses before being included in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki. The protocol was reviewed and approved by the institutional review committee before the study was started. The nature, purpose, and potential risks of the study were explained to each volunteer, and written consent was obtained.

Blood Pressure Measurement

The pressor effect of exogenous challenges of Ang II was measured on the finger using a photoplethysmograph (Finapres, Ohmeda). The measurement technique, which is a noninvasive method measuring digital artery blood pressure continuously through a cuff wrapped around the finger, was first described by Penaz in 1973.[23] The monitor provides beat-to-beat blood pressure values (systolic, diastolic, and mean) and heart rate. This device has been demonstrated to correlate well with intra-arterial pressure[24,25] and to be accurate for the study of short-lasting blood pressure changes by vasoactive agents in humans.[26] Blood pressure and heart rate were continuously recorded on graduated paper 10 minutes before and at least 15 minutes after injection of each Ang II challenge. Peak blood pressure changes were calculated using these tracings.

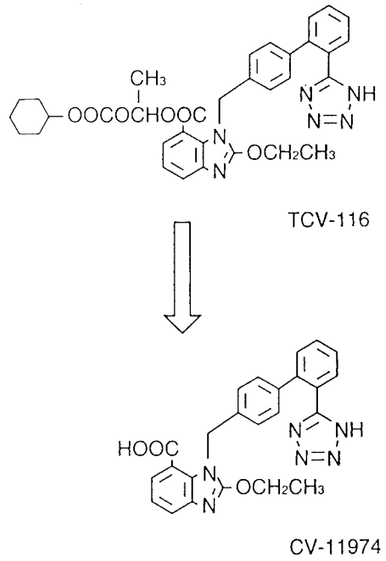

FIG 1. Structural formula of the angiotensin II receptor antagonist TCV-116, or (±)-1-(cyclohexyloxycarbonyloxy)ethyl-2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1-*H*-benzimidazole-7-carboxylate, and its active metabolite, CV-11974.

Study Design

Throughout the study, volunteers were on a free sodium intake. No medication other than the study drug was allowed, neither were cigarette smoking and consumption of alcohol or caffeine-containing beverages and food. One week before the first study day, a dose-response curve to intravenous bolus injections of Ang II was established for each subject. The goal was to obtain a test dose able to increase systolic blood pressure (SBP) by 25 to 40 mm Hg. Ang II (Clinalfa) was dissolved in 0.9% NaCl to achieve a concentration of 1 μg/mL. After a polytetrafluoroethylene cannula was placed in the antecubital vein and after a 30-minute resting period to reach a steady baseline blood pressure and heart rate, bolus injections were started at a dose of 10 ng/kg and increased thereafter every 15 to 20 minutes by increments of 10 ng/kg until the required blood pressure increase was reached. This individual final test dose was then repeated at least twice and was subsequently used to assess the inhibitory effects of TCV-116 during the entire study. The median dose determined for Ang II challenges was 30 ng/kg (range, 10 to 60 ng/kg). The corresponding baseline responses (increase of SBP after Ang II challenges) were on average 31.1 mm Hg (range, 28.3 to 38.7 mm Hg).

TCV-116 [(±)-1-(cyclohexyloxycarbonyloxy)ethyl-2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1-*H*-benzimidazole-7-carboxylate] (Fig 1) is the active metabolite of CV-11974. In fact, CV-11974 was synthesized first, but its bioavailability was less than 5% in animals. Therefore, the prodrug, TCV-116, was developed by esterizing the carboxylic group of CV-11974 at position 7 of the benzimidazole ring (see Fig 1). After oral administration, ^{14}C-labeled TCV-116 is not detected in plasma, whereas radiolabeled CV-11974 is present.[27] In vitro studies have shown that the inhibitory effect of TCV-116 on the Ang II (10^{-8} mol/L)–induced contraction of rabbit aorta was approximately one thirtieth that of CV-11974.[27] Accordingly, it appears that the inhibitory activity resides almost exclusively in CV-11974. TCV-116 is absorbed and converted to the active metabolite CV-11974 (Fig 1). Results of in vitro studies with ^{14}C-labeled CV-11974 showed that the protein binding of the compound was concentration independent over the range of 10 to 10^4 ng/mL. In rats, dogs, and humans, 99.6% to 99.8%, 96.7% to 97.5%, and 99.4% to 99.6% of CV-11974 is bound to protein.

TCV-116 was provided by Takeda Europe R&D Centre in the form of 1-, 2-, 4-, and 8-mg film-coated tablets. Placebo tablets were identical in appearance. The tablets were administered with 200 mL tap water.

Volunteers received on the mornings of 8 consecutive days one dose of TCV-116 (1, 2, or 4 mg) or placebo. Thus, each dose or placebo was given in a double-blind fashion to 6 volunteers, except the 1-mg dose, which was given to only 5 volunteers because of a dropout at entry screening. The 8-mg dose was administered subsequently to 4 additional volunteers in a single-blind fashion, when it had become evident that doses up to 4 mg may not produce maximal inhibition of the Ang II pressor response. During study days 1 through 9, the volunteers came every day at 6:30 AM to the research facility having fasted overnight (from 10 PM). Supine blood pressure and heart rate were measured after a 45-minute supine resting period. On study days 1 through 8, the dose of TCV-116 or placebo was administered at 8 AM. On days 1 and 8, systolic and diastolic pressor effects of Ang II challenges were measured before and after drug or placebo intake. Venous cannulas were inserted into a vein in each forearm, one line for angiotensin injections and the other for blood sampling. The blood pressure–monitoring cuff was wrapped around the third or fourth finger on the side used for blood sampling. After a period of bed rest of at least 45 minutes and approximately 45 minutes before TCV-116 or placebo was given to the volunteers, the established test dose of Ang II (selected according to the predetermined dose-response curve) was administered. The effect of the drug was then monitored on days 1 and 8 using bolus injections of the Ang II test dose at 1, 2, 3, 4, 6, 8, 12, 24, and 36 hours after drug intake. Additional Ang II challenges were also performed on day 4 before and 2, 6, and 12 hours after TCV-116 or placebo administration. The volunteers remained fasting and in a supine position for 6 hours after drug administration; they then received a light meal and remained resting in bed or seated for 6 more hours (supine for 1 hour before each blood sampling). Between hours 12 and 22.5, they were allowed to leave the hospital. At the end of the study, all volunteers underwent routine and laboratory safety evaluation.

Hormone and Drug Measurements

On days 1 and 8, plasma renin activity (PRA), Ang II, aldosterone, norepinephrine, and epinephrine were measured immediately before and 2, 4, 6, 12, and 24 hours after drug or placebo intake. The same parameters were also measured on day 4 immediately before drug or placebo intake. Additional samples for the measurement of plasma concentrations of CV-11974, the active metabolite of TCV-116, were drawn on every day of the study immediately before drug intake and repeatedly on days 1 and 8 (days 1 and 8: 1, 2, 2.5, 3, 3.5, 4, 6, 8, 12, and 24 hours; day 8: 30 and 36 hours after drug or placebo intake). Blood sampling was always performed immediately before the next angiotensin challenge. Volunteers remained in a supine position for 60 minutes before all these blood samples.

Blood samples (5 mL) for measurement of plasma CV-11974 concentration were collected into heparinized tubes, and plasma was stored at −70°C until analyzed. The active metabolite CV-11974 (molecular weight, 610.67) was analyzed by reversed-phase high-performance liquid chromatography (HPLC) after extraction from acidified serum into ethyl ether.

Systolic Blood Pressure Response to Angiotensin II, Plasma CV-11974 and Angiotensin II Concentrations, and Plasma Renin Activity Before and After Administration of TCV-116 or Placebo to Healthy Volunteers on Days 1 and 8

Treatment	Time After Drug Intake, h	SBP, % of Day 1 Baseline		CV-11974, ng/mL		Ang II, fmol/mL		PRA, (ng/mL)/h	
		Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
Placebo (n=6)	0	100±0	100±11	0±0	0±0	4.5±0.8	5.3±1.2	0.79±0.22	0.91±0.20
	2	104±4	104±5	0.2±0.1	0±0	5.5±0.7	6.4±1.5	0.83±0.22	1.07±0.33
	4	97±7	100±4	0.3±0.1	0±0	4.8±0.7	5.4±0.8	0.82±0.16	0.83±0.26
	6	99±9	106±7	0±0	0±0	6.3±1.0	7.2±1.5	1.05±0.29	1.29±0.35
	24	101±5	99±7	0±0	0±0	5.0±0.9	4.0±0.7	0.77±0.15	0.81±0.18
TCV-116, 1 mg (n=5)	0	100±0	83±8	0±0	0±0	3.2±0.6	5.9±1.0	0.65±0.1	1.26±0.20
	2	93±8	71±7	4.2±0.5	5.3±1.0	3.9±1.1	7.3±1.5	0.79±0.17	1.50±0.28
	4	89±11	64±9	5.5±0.3	8.8±1.2	5.6±1.0	10.2±3.4	1.17±0.25	2.10±0.60
	6	82±9	51±12	4.4±0.5	7.8±1.0	8.2±2.1	11.5±3.0	1.63±0.49	2.14±0.56
	24	94±9	84±9	0±0	0.4±0.3	5.5±1.1	6.7±1.6	1.26±0.23	1.55±0.32
TCV-116, 2 mg (n=6)	0	100±0	82±11	0±0	1.5±0.5	3.9±0.8	8.1±1.1	0.71±0.19	1.52±0.17
	2	85±9	74±10	7.6±2.4	12.4±2.1	7.6±2.1	16.3±3.0	1.39±0.33	3.13±0.43
	4	82±11	58±8	12.7±1.6	17.6±2.4	17.8±6.2	14.5±1.5	3.18±1.03	3.17±0.40
	6	67±9	57±10	10.9±0.8	14.2±1.7	20.8±6.4	22.2±3.2	3.93±1.13	4.75±0.45
	24	75±5	70±9	0.9±0.3	1.8±0.3	10.7±3.0	12.1±2.5	2.14±0.61	2.62±0.43
TCV-116, 4 mg (n=6)	0	100±0	66±8	0±0	3.3±0.9	5.3±1.4	20.6±5.1	0.90±0.26	3.93±0.80
	2	87±8	51±13	23.5±2.7	23.9±3.3	7.9±1.9	31.9±7.0	1.33±0.31	5.98±1.28
	4	41±9	21±7	34.3±3.9	42.8±9.1	19.9±4.9	57.4±14.3	3.99±1.21	11.72±4.06
	6	40±4	35±8	27.7±3.5	42.8±12.5	21.3±4.7	57.0±7.7	3.87±0.86	11.98±2.53
	24	58±11	53±9	2.2±0.4	4.0±1.3	10.1±2.6	13.9±2.2	1.99±0.53	3.28±0.57
TCV-116, 8 mg (n=4)	0	100±0	44±16	0±0	3.9±1.8	3.0±0.7	14.2±5.5	0.35±0.25	2.85±1.06
	2	52±9	36±14	36.8±7.1	34.1±5.9	10.1±2.8	30.9±14.5	1.74±0.52	5.85±2.46
	4	22±3	16±6	47.4±6.4	48.2±7.0	15.9±4.0	39.4±20.7	2.53±0.36	6.60±2.88
	6	19±5	15±9	33.3±4.5	36.3±5.8	22.1±6.5	55.4±25.1	4.18±1.20	7.25±3.04
	24	49±6	53±13	3.8±0.5	4.9±0.8	12.1±2.0	11.6±0.7	2.35±0.38	2.73±0.48

SBP indicates systolic blood pressure; Ang, angiotensin; and PRA, plasma renin activity. For CV-11974, 1 ng/mL=1.64 nmol/L; for PRA, 1 (ng/mL)/h=0.77 (nmol/L)/h.

Briefly, 0.5 mL serum was acidified with 0.5 mL of 0.2 mol/L hydrochloric acid. After mixing, 5.0 mL ethyl ether was added to extract CV-11974 by shaking for 15 minutes. One hundred microliters of 10% propylene glycol in methanol was added to the organic extract before evaporation under nitrogen. The residue was taken up in 200 μL of mobile phase A (acetonitrile/KH_2PO_4 [20 mmol/L], pH adjusted to 3.5 with 85% H_3PO_4). One hundred microliters was injected. A column-switching HPLC method was used. A fraction containing CV-11974 from column A was eluted by mobile phase A into column B. CV-11974 was separated from the coeluting endogenous compounds using mobile phase B (acetonitrile/KH_2PO_4 [20 mmol/L], 34:66). A 10-port column-switching valve was used to control the time events. The detection limit of CV-11974 was 0.8 ng/mL or 1.3 nmol/L in human serum. The extraction recovery of CV-11974 from serum was 70% and was consistent over the entire standard curve range.

For the measurement of PRA, generated Ang I was trapped and quantitated by high-affinity antibodies.[28,29] Immunoreactive Ang II was quantitatively extracted from plasma by reversible adsorption to phenylsilyl silica and estimated by radioimmunoassay using monoclonal antibodies against Ang II.[30] Plasma aldosterone was determined by a direct radioimmunoassay.[31] Plasma norepinephrine and epinephrine levels were determined using the radioenzymatic method of Peuler and Johnson[32] as modified for our laboratory.[33] Subjects remained in a supine position for 30 minutes before blood sampling.

The percent of baseline pressor response to Ang II was time-integrated up to 24 hours in calculating the area under the curve according to a trapezoidal rule for each individual volunteer. The same was done for time integration of CV-11974 concentration and PRA and Ang II plasma levels up to 24 hours.

Pharmacokinetic Calculations

A one-compartment model after extravascular administration was fitted to the plasma data by extended least-squares nonlinear regression with the error model=v(1) (homoscedastic model). In two subjects, a two-compartment model had to be used. The area under the time versus concentration time curve (AUC) was calculated using the trapezoidal rule in the ascending portion of the curve and the log-trapezoidal rule for the descending concentrations and was extrapolated to infinity at day 1 and up to 24 hours at day 8 (one dosing interval). The (apparent) clearance (CL′) was calculated assuming complete absorption and transformation of TCV-116 into CV-11974 as dose/AUC and the terminal half-life ($t_{1/2}$) as $Ln(2)/\lambda_z$. The mean residence time (MRT) was calculated as (AUMC/AUC)−MAT, where AUMC represents the area under the first moment of the concentration versus time curve (to infinity) and MAT the mean formation time.

Statistical Analysis

All values are mean±SEM. Blood pressure and heart rate responses to the Ang II challenge were defined as the difference between the values before and after individual challenges and expressed as percent of the mean baseline response to the individual final test dose of Ang II. Statistical analysis was performed using ANOVA for repeated measures and paired *t* test with the Bonferroni adjustment for multiple comparisons (SUPERANOVA 1.1, Abacus Concepts, Inc). The time-integrated parameters (SBP response to Ang II, PRA, plasma drug, and Ang II concentrations) were analyzed by a two-factor ANOVA followed when required by a Fischer's protected least significant difference test. The correlation coefficients were calculated when indicated by the least-squares method. A probability value of less than .05 was considered significant.

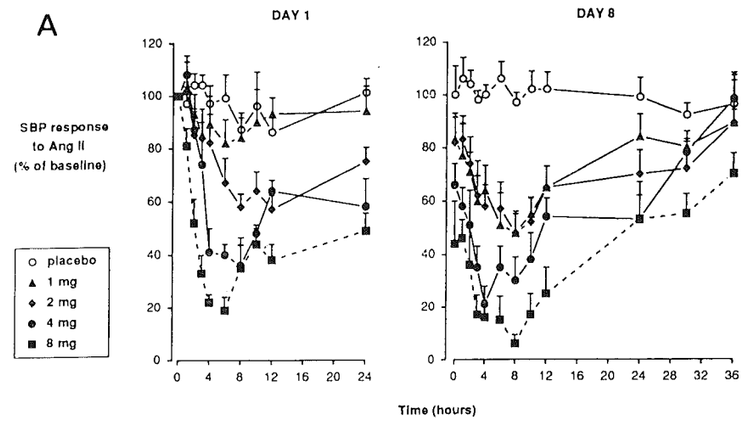

FIG 2. A, Line graphs show effect of 8 consecutive days of treatment (days 1 [left] and 8 [right]) with four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) or placebo on systolic blood pressure (SBP) response to individually predetermined test dose of angiotensin II (Ang II) in healthy volunteers (mean±SEM). Baseline response (100%) was determined before first drug or placebo administration. B, Bar graphs show time integral of inhibition of pressor response to Ang II challenges on days 1 (left) and 8 (right) of treatment with placebo or 1, 2, 4, and 8 mg/d PO TCV-116. *P<.05, †P<.01.

Results

Safety of Oral Administration of TCV-116

No clinically significant adverse reaction was observed in any volunteer during the study. TCV-116 had no effect on (resting) supine or upright blood pressure and heart rate after the first administration or during the 8-day treatment. TCV-116 did not modify blood cell counts, routine laboratory tests, urine analyses, or electrocardiograms. Of the 24 volunteers collected for the initial randomization, 2 had to be withdrawn before administration of the medication because of abnormal laboratory findings and only 1 of them could be replaced in time.

Blood Pressure Response to Ang II

The Table and Fig 2 show the dose-related inhibition of the SBP response to exogenous Ang II. Doses of 1, 2, 4, and 8 mg induced a dose-related inhibition of the response to Ang II. On day 1, the peak inhibitory effect was reached between 4 and 8 hours after drug intake; 4 hours after the 4-mg dose, the blood pressure response to Ang II decreased to 41±9% and 21±7% of the baseline response on days 1 and 8, respectively. The mean blood pressure response to Ang II of the 4 volunteers treated with the 8-mg dose in a single-blind fashion decreased to 22±3% and 16±6% 4 hours after drug intake. On day 1, a significant attenuation of the blood pressure response to Ang II was still present 24 hours after intake of the 2-, 4-, and 8-mg doses of TCV-116 (75±5%, P<.01; 58±11%, P<.05; 49±6%, P<.01, respectively, versus predrug blood pressure response). The integral of SBP response over 24 hours is represented in Fig 2B. The trough effect on day 8 showed no statistically significant difference compared with the trough effect on day 1. Except for the 1-mg dose on day 1, any dose of TCV-116 significantly reduced the SBP response to Ang II, and this reduction was dose related. The time-integrated SBP response tended to decrease slightly more on day 8 than on day 1.

Pharmacokinetics

Plasma concentrations of TCV-116 were not detected. After the administration of TCV-116, its active metabolite CV-11974 appeared after a mean lag time of 1.0 hour (days 1 and 8) and reached a peak (T_{max}) between 3.5 and 6 hours. Its mean formation time was 1.2 and 1.3 hours on days 1 and 8, respectively. The mean concentrations of CV-11974 are described in the Table and plotted in Fig 3 (day 1: Fig 3A, left; day 8: Fig 3A, right). Maximal concentrations (T_{max}) and AUC values increased in proportion to the dose after the three low doses but less than expected at the high dose. Except once in 2 subjects, the plasma concentrations declined monoexponentially with half-life periods of 3.5 hours (day 1) and 4.0 hours (day 8). Plasma levels were still measurable at 24 hours, and trough concentrations remained unchanged from day 2 through day 8 for all doses of TCV-116. MRT values were 8.1 and 9.7 hours on days 1 and 8, respectively. The apparent clearance of CV-11974, its maximal possible clearance given the assumptions made in its calculation, were 0.25 $L \cdot h^{-1} \cdot kg^{-1}$ on day 1 and 0.20 $L \cdot h^{-1} \cdot kg^{-1}$ on day 8.

Dose-Effect Relations

In Fig 4A, the mean inhibition of the pressure response to exogenous Ang II challenge is plotted against the respective concentrations of CV-11974 for each dose and each time point (up to 24 hours) on day 1. The dose-effect relation shows a considerable dispersion of

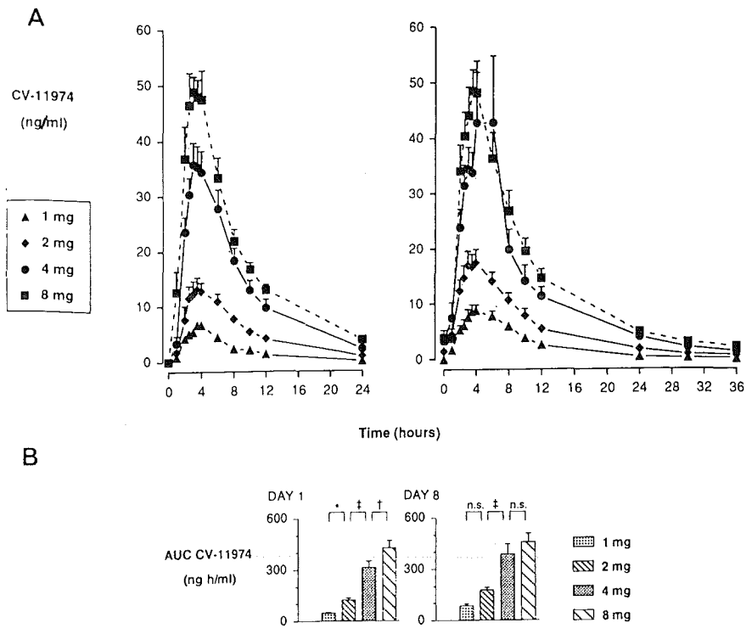

FIG 3. A, Line graphs show time profile of mean plasma concentrations of CV-11974, the active metabolite of TCV-116, on days 1 (left) and 8 (right) treatment with four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) (mean±SEM). B, Bar graphs show area under the concentration-time curve (AUC) of the active metabolite CV-11974 on days 1 (left) and 8 (right) of treatment with 1, 2, 4, and 8 mg/d PO TCV-116. *$P<.05$, †$P<.01$, ‡$P<.001$.

individual values (not shown) and mean values (Fig 4A, left), which accounts for an anticlockwise hysteresis loop (Fig 4A, right) on day 1. This loop characterizes a slow onset of the inhibitory effect of the drug on blood pressure while plasma concentrations of CV-11974 are increasing, and a sustained effect when drug concentrations are falling. The time necessary to collapse both arms of the curve varies between 1 and 2 hours. This

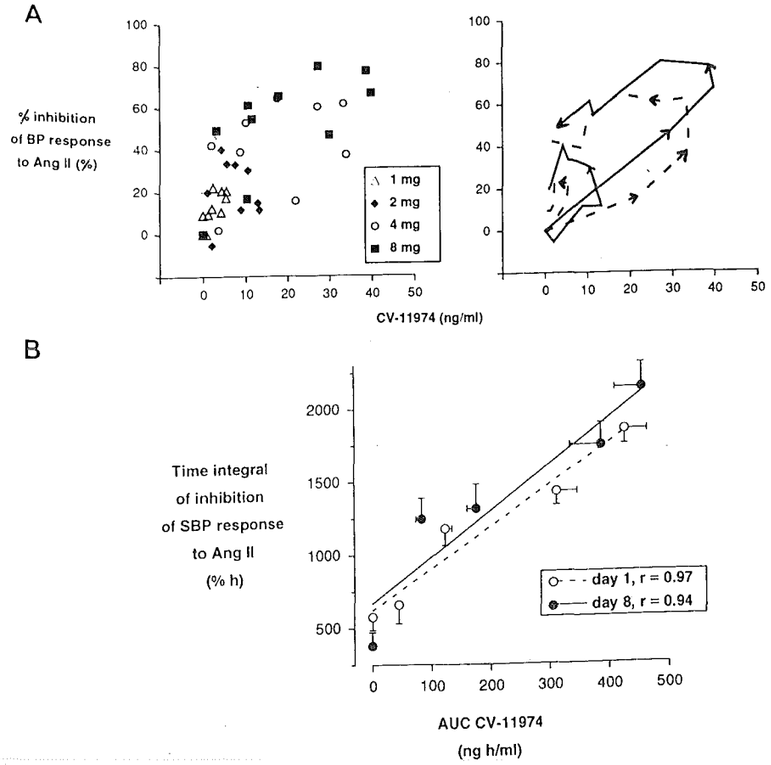

FIG 4. A, left, Scatterplot shows mean CV-11974 concentrations vs mean inhibition of systolic blood pressure (BP) response to angiotensin II (Ang II) after four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) on day 1. Right, Points are connected in a time-ascending fashion for each dose. B, Line graph shows time integral of the inhibition of the pressor response to Ang II challenge vs the area under the concentration-time curve (AUC) of the metabolite in 27 volunteers on days 1 and 8 of an 8-day treatment with placebo or 1, 2, 4, and 8 mg/d PO TCV-116. Dashed and solid lines represent the linear correlation of days 1 and 8, respectively. SBP indicates systolic blood pressure.

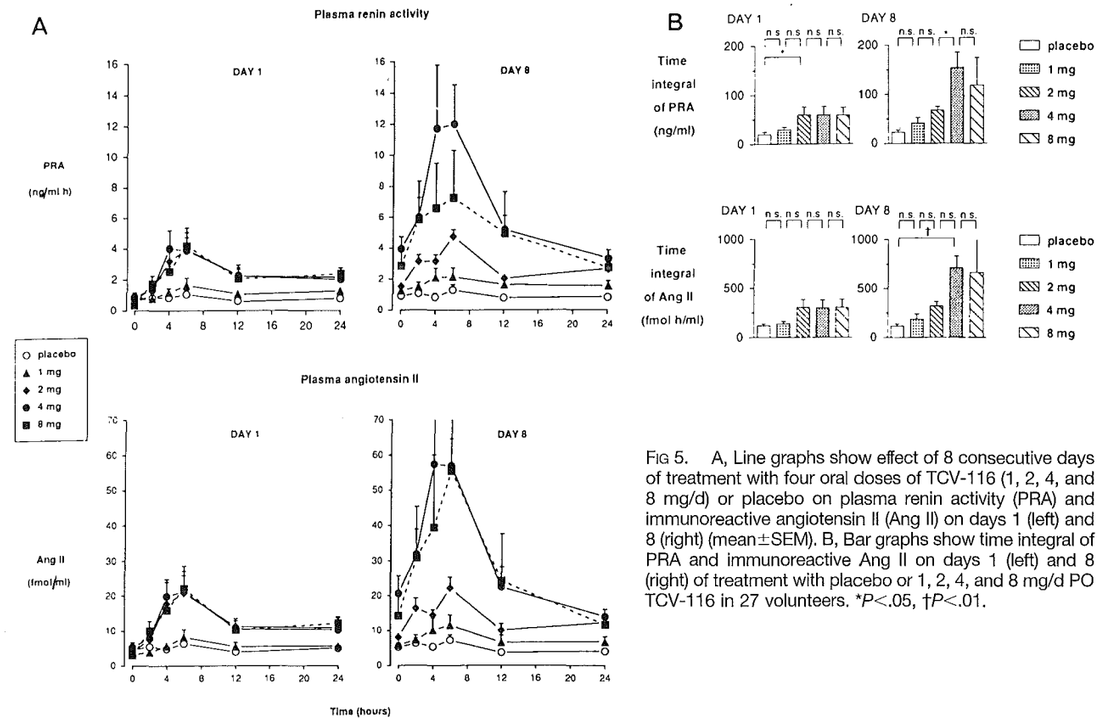

FIG 5. A, Line graphs show effect of 8 consecutive days of treatment with four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) or placebo on plasma renin activity (PRA) and immunoreactive angiotensin II (Ang II) on days 1 (left) and 8 (right) (mean±SEM). B, Bar graphs show time integral of PRA and immunoreactive Ang II on days 1 (left) and 8 (right) of treatment with placebo or 1, 2, 4, and 8 mg/d PO TCV-116 in 27 volunteers. *$P<.05$, †$P<.01$.

loop in dose-effect relations exists for each individual volunteer (data not shown). On day 8, the loop is flattened (data not shown). Furthermore, for a given plasma drug level, the degree of inhibition of the SBP response to Ang II seems to be higher on day 8 than on day 1. Fig 4B illustrates the same relation for the time-integrated data of individual subjects on days 1 and 8 taking into account the duration of the drug effect (the integral of the percentage of inhibition over 24 hours was related to the AUC of CV-11974 during the same period). This time-integrated dose-effect relation showed a linear pattern without any plateau, although the maximal inhibition is reached with the higher CV-11974 plasma concentrations. Although the magnitude of the inhibition of the blood pressure response to Ang II does not change, its duration is prolonged, leading to a linear increase of the time-integrated hemodynamic effect.

Neurohumoral Variables

The Table and Fig 5 depict PRA and Ang II plasma levels measured at the first and eighth administration of placebo or TCV-116. Both PRA and Ang II showed a marked dose-related increase 6 hours after drug intake, and this increase reached clearly higher values on day 8 than on day 1. Both variables had already increased significantly 4 hours after administration of 2, 4, and 8 mg TCV-116 on the first day ($P<.05$ versus placebo).

A very close correlation was found between PRA and plasma Ang II ($r=.91$, n=348, $P<.001$). There was also a negative correlation between the increase in PRA and the SBP response to exogenous Ang II ($r=-.456$, n=348, $P<.001$). Neither plasma norepinephrine nor plasma epinephrine (not shown) changed during the 8-day administration of TCV-116.

Fig 6 shows plasma aldosterone concentrations. Plasma aldosterone levels decreased after administration of single doses of TCV-116, but a similar decrease was also seen after placebo, reflecting the circadian variation in aldosterone concentrations.

Discussion

The present data demonstrate that TCV-116 is a potent, orally active Ang II antagonist with a relatively long duration of action. At peak effect (8 hours after drug intake), the 4-mg dose induced a 72±5% and 77±4% reduction in the systolic and diastolic blood pressure responses to Ang II, respectively. With the three higher doses used, ie, 2, 4, and 8 mg, a definite blocking effect was still present 24 hours after drug intake (day 1). With these three higher doses, the trough inhibition of Ang II pressor effect was greater on day 8 than on day 1, so that before drug intake on day 8, the pressor response to Ang II was only approximately 75% of the comparable response on day 1, suggesting that the drug exerts an effect that lasts for more than 24 hours. With repeated administration, no significant cumulative progressive enhancement of the blocking action could be observed at peak or trough. The dose dependency of the hemodynamic effect was clearly demonstrated with the four doses of TCV-116 throughout the treatment period (Fig 2).

The presence of constant trough levels from day 2 to day 8 suggests that no accumulation is occurring. These residual levels account for a negligible fraction (<6%) of the AUC values. The absence of a dose-proportional increase

Systolic Blood Pressure Response to Angiotensin II, Plasma CV-11974 and Angiotensin II Concentrations, and Plasma Renin Activity Before and After Administration of TCV-116 or Placebo to Healthy Volunteers on Days 1 and 8

Treatment	Time After Drug Intake, h	SBP, % of Day 1 Baseline		CV-11974, ng/mL		Ang II, fmol/mL		PRA, (ng/mL)/h	
		Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
Placebo (n=6)	0	100±0	100±11	0±0	0±0	4.5±0.8	5.3±1.2	0.79±0.22	0.91±0.20
	2	104±4	104±5	0.2±0.1	0±0	5.5±0.7	6.4±1.5	0.83±0.22	1.07±0.33
	4	97±7	100±4	0.3±0.1	0±0	4.8±0.7	5.4±0.8	0.82±0.16	0.83±0.26
	6	99±9	106±7	0±0	0±0	6.3±1.0	7.2±1.5	1.05±0.29	1.29±0.35
	24	101±5	99±7	0±0	0±0	5.0±0.9	4.0±0.7	0.77±0.15	0.81±0.18
TCV-116, 1 mg (n=5)	0	100±0	83±8	0±0	0±0	3.2±0.6	5.9±1.0	0.65±0.1	1.26±0.20
	2	93±8	71±7	4.2±0.5	5.3±1.0	3.9±1.1	7.3±1.5	0.79±0.17	1.50±0.28
	4	89±11	64±9	5.5±0.3	8.8±1.2	5.6±1.0	10.2±3.4	1.17±0.25	2.10±0.60
	6	82±9	51±12	4.4±0.5	7.8±1.0	8.2±2.1	11.5±3.0	1.63±0.49	2.14±0.56
	24	94±9	84±9	0±0	0.4±0.3	5.5±1.1	6.7±1.6	1.26±0.23	1.55±0.32
TCV-116, 2 mg (n=6)	0	100±0	82±11	0±0	1.5±0.5	3.9±0.8	8.1±1.1	0.71±0.19	1.52±0.17
	2	85±9	74±10	7.6±2.4	12.4±2.1	7.6±2.1	16.3±3.0	1.39±0.33	3.13±0.43
	4	82±11	58±8	12.7±1.6	17.6±2.4	17.8±6.2	14.5±1.5	3.18±1.03	3.17±0.40
	6	67±9	57±10	10.9±0.8	14.2±1.7	20.8±6.4	22.2±3.2	3.93±1.13	4.75±0.45
	24	75±5	70±9	0.9±0.3	1.8±0.3	10.7±3.0	12.1±2.5	2.14±0.61	2.62±0.43
TCV-116, 4 mg (n=6)	0	100±0	66±8	0±0	3.3±0.9	5.3±1.4	20.6±5.1	0.90±0.26	3.93±0.80
	2	87±8	51±13	23.5±2.7	23.9±3.3	7.9±1.9	31.9±7.0	1.33±0.31	5.98±1.28
	4	41±9	21±7	34.3±3.9	42.8±9.1	19.9±4.9	57.4±14.3	3.99±1.21	11.72±4.06
	6	40±4	35±8	27.7±3.5	42.8±12.5	21.3±4.7	57.0±7.7	3.87±0.86	11.98±2.53
	24	58±11	53±9	2.2±0.4	4.0±1.3	10.1±2.6	13.9±2.2	1.99±0.53	3.28±0.57
TCV-116, 8 mg (n=4)	0	100±0	44±16	0±0	3.9±1.8	3.0±0.7	14.2±5.5	0.35±0.25	2.85±1.06
	2	52±9	36±14	36.8±7.1	34.1±5.9	10.1±2.8	30.9±14.5	1.74±0.52	5.85±2.46
	4	22±3	16±6	47.4±6.4	48.2±7.0	15.9±4.0	39.4±20.7	2.53±0.36	6.60±2.88
	6	19±5	15±9	33.3±4.5	36.3±5.8	22.1±6.5	55.4±25.1	4.18±1.20	7.25±3.04
	24	49±6	53±13	3.8±0.5	4.9±0.8	12.1±2.0	11.6±0.7	2.35±0.38	2.73±0.48

SBP indicates systolic blood pressure; Ang, angiotensin; and PRA, plasma renin activity. For CV-11974, 1 ng/mL=1.64 nmol/L; for PRA, 1 (ng/mL)/h=0.77 (nmol/L)/h.

Briefly, 0.5 mL serum was acidified with 0.5 mL of 0.2 mol/L hydrochloric acid. After mixing, 5.0 mL ethyl ether was added to extract CV-11974 by shaking for 15 minutes. One hundred microliters of 10% propylene glycol in methanol was added to the organic extract before evaporation under nitrogen. The residue was taken up in 200 μL of mobile phase A (acetonitrile/KH_2PO_4 [20 mmol/L], pH adjusted to 3.5 with 85% H_3PO_4). One hundred microliters was injected. A column-switching HPLC method was used. A fraction containing CV-11974 from column A was eluted by mobile phase A into column B. CV-11974 was separated from the coeluting endogenous compounds using mobile phase B (acetonitrile/KH_2PO_4 [20 mmol/L], 34:66). A 10-port column-switching valve was used to control the time events. The detection limit of CV-11974 was 0.8 ng/mL or 1.3 nmol/L in human serum. The extraction recovery of CV-11974 from serum was 70% and was consistent over the entire standard curve range.

For the measurement of PRA, generated Ang I was trapped and quantitated by high-affinity antibodies.[28,29] Immunoreactive Ang II was quantitatively extracted from plasma by reversible adsorption to phenylsilyl silica and estimated by radioimmunoassay using monoclonal antibodies against Ang II.[30] Plasma aldosterone was determined by a direct radioimmunoassay.[31] Plasma norepinephrine and epinephrine levels were determined using the radioenzymatic method of Peuler and Johnson[32] as modified for our laboratory.[33] Subjects remained in a supine position for 30 minutes before blood sampling.

The percent of baseline pressor response to Ang II was time-integrated up to 24 hours in calculating the area under the curve according to a trapezoidal rule for each individual volunteer. The same was done for time integration of CV-11974 concentration and PRA and Ang II plasma levels up to 24 hours.

Pharmacokinetic Calculations

A one-compartment model after extravascular administration was fitted to the plasma data by extended least-squares nonlinear regression with the error model=v(1) (homoscedastic model). In two subjects, a two-compartment model had to be used. The area under the time versus concentration time curve (AUC) was calculated using the trapezoidal rule in the ascending portion of the curve and the log-trapezoidal rule for the descending concentrations and was extrapolated to infinity at day 1 and up to 24 hours at day 8 (one dosing interval). The (apparent) clearance (CL′) was calculated assuming complete absorption and transformation of TCV-116 into CV-11974 as dose/AUC and the terminal half-life ($t_{1/2}$) as $Ln(2)/\lambda_z$. The mean residence time (MRT) was calculated as (AUMC/AUC)−MAT, where AUMC represents the area under the first moment of the concentration versus time curve (to infinity) and MAT the mean formation time.

Statistical Analysis

All values are mean±SEM. Blood pressure and heart rate responses to the Ang II challenge were defined as the difference between the values before and after individual challenges and expressed as percent of the mean baseline response to the individual final test dose of Ang II. Statistical analysis was performed using ANOVA for repeated measures and paired *t* test with the Bonferroni adjustment for multiple comparisons (SUPERANOVA 1.1, Abacus Concepts, Inc). The time-integrated parameters (SBP response to Ang II, PRA, plasma drug, and Ang II concentrations) were analyzed by a two-factor ANOVA followed when required by a Fischer's protected least significant difference test. The correlation coefficients were calculated when indicated by the least-squares method. A probability value of less than .05 was considered significant.

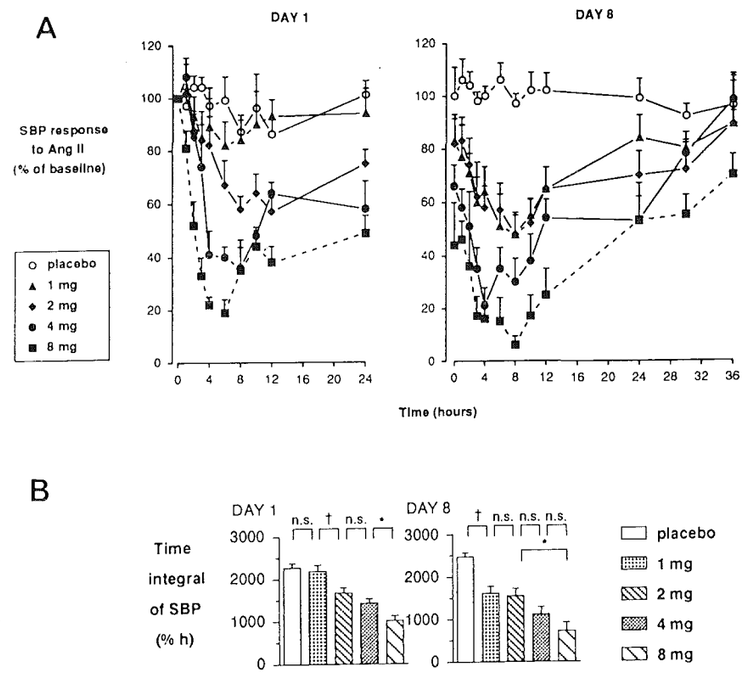

FIG 2. A, Line graphs show effect of 8 consecutive days of treatment (days 1 [left] and 8 [right]) with four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) or placebo on systolic blood pressure (SBP) response to individually predetermined test dose of angiotensin II (Ang II) in healthy volunteers (mean±SEM). Baseline response (100%) was determined before first drug or placebo administration. B, Bar graphs show time integral of inhibition of pressor response to Ang II challenges on days 1 (left) and 8 (right) of treatment with placebo or 1, 2, 4, and 8 mg/d PO TCV-116. *$P<.05$, †$P<.01$.

Results

Safety of Oral Administration of TCV-116

No clinically significant adverse reaction was observed in any volunteer during the study. TCV-116 had no effect on (resting) supine or upright blood pressure and heart rate after the first administration or during the 8-day treatment. TCV-116 did not modify blood cell counts, routine laboratory tests, urine analyses, or electrocardiograms. Of the 24 volunteers collected for the initial randomization, 2 had to be withdrawn before administration of the medication because of abnormal laboratory findings and only 1 of them could be replaced in time.

Blood Pressure Response to Ang II

The Table and Fig 2 show the dose-related inhibition of the SBP response to exogenous Ang II. Doses of 1, 2, 4, and 8 mg induced a dose-related inhibition of the response to Ang II. On day 1, the peak inhibitory effect was reached between 4 and 8 hours after drug intake; 4 hours after the 4-mg dose, the blood pressure response to Ang II decreased to 41±9% and 21±7% of the baseline response on days 1 and 8, respectively. The mean blood pressure response to Ang II of the 4 volunteers treated with the 8-mg dose in a single-blind fashion decreased to 22±3% and 16±6% 4 hours after drug intake. On day 1, a significant attenuation of the blood pressure response to Ang II was still present 24 hours after intake of the 2-, 4-, and 8-mg doses of TCV-116 (75±5%, $P<.01$; 58±11%, $P<.05$; 49±6%, $P<.01$, respectively, versus predrug blood pressure response). The integral of SBP response over 24 hours is represented in Fig 2B. The trough effect on day 8 showed no statistically significant difference compared with the trough effect on day 1. Except for the 1-mg dose on day 1, any dose of TCV-116 significantly reduced the SBP response to Ang II, and this reduction was dose related. The time-integrated SBP response tended to decrease slightly more on day 8 than on day 1.

Pharmacokinetics

Plasma concentrations of TCV-116 were not detected. After the administration of TCV-116, its active metabolite CV-11974 appeared after a mean lag time of 1.0 hour (days 1 and 8) and reached a peak (T_{max}) between 3.5 and 6 hours. Its mean formation time was 1.2 and 1.3 hours on days 1 and 8, respectively. The mean concentrations of CV-11974 are described in the Table and plotted in Fig 3 (day 1: Fig 3A, left; day 8: Fig 3A, right). Maximal concentrations (T_{max}) and AUC values increased in proportion to the dose after the three low doses but less than expected at the high dose. Except once in 2 subjects, the plasma concentrations declined monoexponentially with half-life periods of 3.5 hours (day 1) and 4.0 hours (day 8). Plasma levels were still measurable at 24 hours, and trough concentrations remained unchanged from day 2 through day 8 for all doses of TCV-116. MRT values were 8.1 and 9.7 hours on days 1 and 8, respectively. The apparent clearance of CV-11974, its maximal possible clearance given the assumptions made in its calculation, were 0.25 $L \cdot h^{-1} \cdot kg^{-1}$ on day 1 and 0.20 $L \cdot h^{-1} \cdot kg^{-1}$ on day 8.

Dose-Effect Relations

In Fig 4A, the mean inhibition of the pressure response to exogenous Ang II challenge is plotted against the respective concentrations of CV-11974 for each dose and each time point (up to 24 hours) on day 1. The dose-effect relation shows a considerable dispersion of

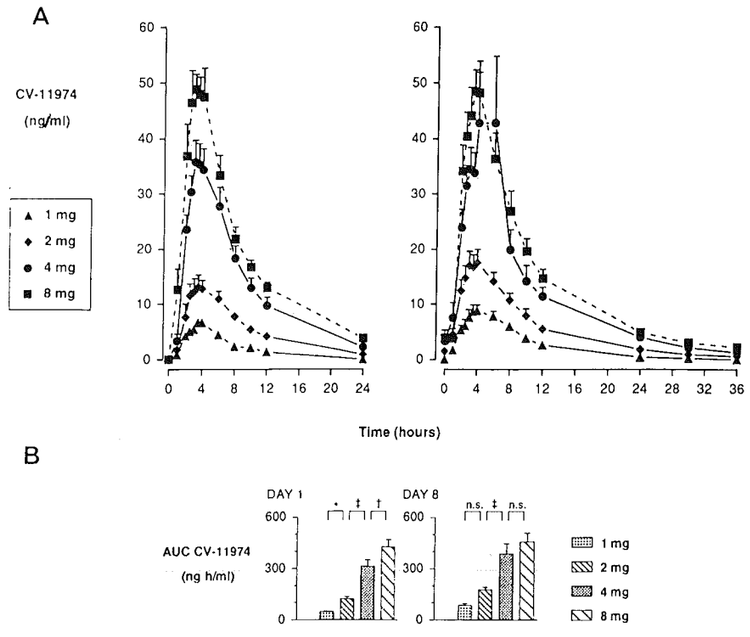

FIG 3. A, Line graphs show time profile of mean plasma concentrations of CV-11974, the active metabolite of TCV-116, on days 1 (left) and 8 (right) treatment with four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) (mean±SEM). B, Bar graphs show area under the concentration-time curve (AUC) of the active metabolite CV-11974 on days 1 (left) and 8 (right) of treatment with 1, 2, 4, and 8 mg/d PO TCV-116. *$P<.05$, †$P<.01$, ‡$P<.001$.

individual values (not shown) and mean values (Fig 4A, left), which accounts for an anticlockwise hysteresis loop (Fig 4A, right) on day 1. This loop characterizes a slow onset of the inhibitory effect of the drug on blood pressure while plasma concentrations of CV-11974 are increasing, and a sustained effect when drug concentrations are falling. The time necessary to collapse both arms of the curve varies between 1 and 2 hours. This

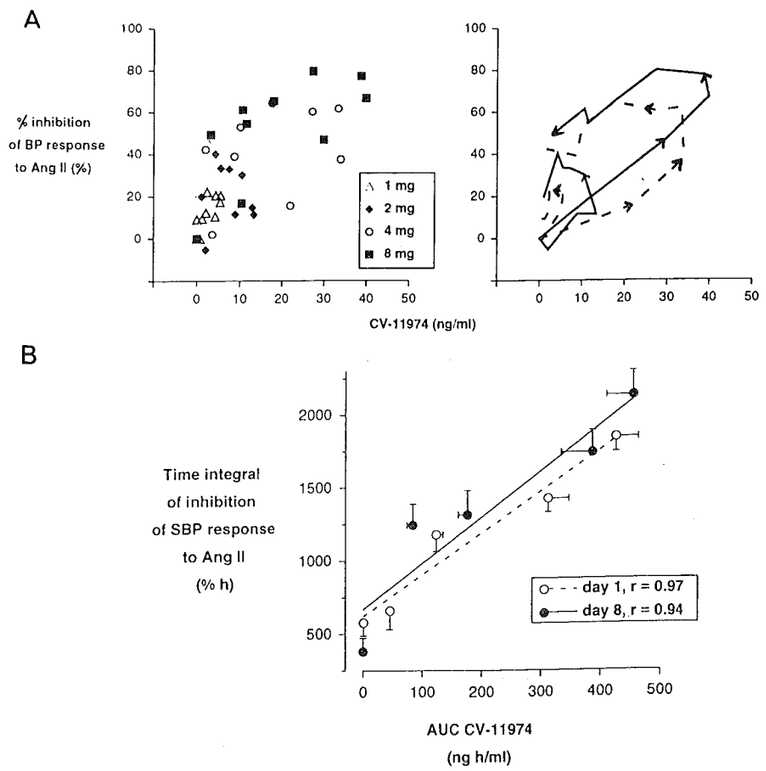

FIG 4. A, left, Scatterplot shows mean CV-11974 concentrations vs mean inhibition of systolic blood pressure (BP) response to angiotensin II (Ang II) after four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) on day 1. Right, Points are connected in a time-ascending fashion for each dose. B, Line graph shows time integral of the inhibition of the pressor response to Ang II challenge vs the area under the concentration-time curve (AUC) of the metabolite in 27 volunteers on days 1 and 8 of an 8-day treatment with placebo or 1, 2, 4, and 8 mg/d PO TCV-116. Dashed and solid lines represent the linear correlation of days 1 and 8, respectively. SBP indicates systolic blood pressure.

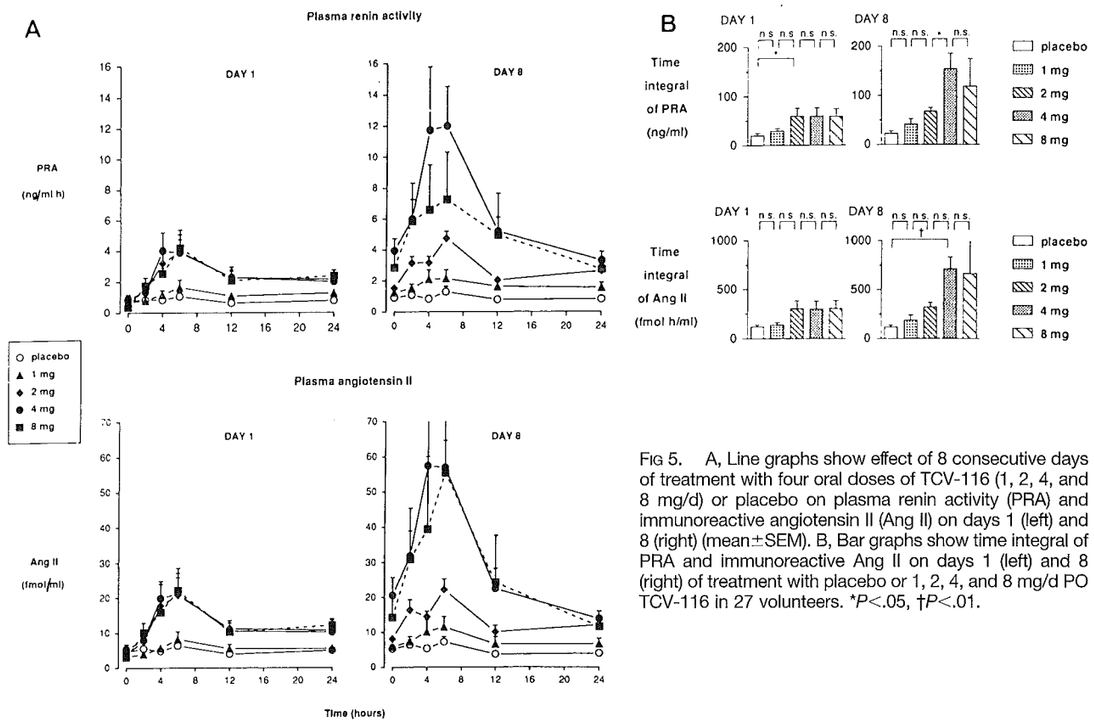

FIG 5. A, Line graphs show effect of 8 consecutive days of treatment with four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) or placebo on plasma renin activity (PRA) and immunoreactive angiotensin II (Ang II) on days 1 (left) and 8 (right) (mean±SEM). B, Bar graphs show time integral of PRA and immunoreactive Ang II on days 1 (left) and 8 (right) of treatment with placebo or 1, 2, 4, and 8 mg/d PO TCV-116 in 27 volunteers. *$P<.05$, †$P<.01$.

loop in dose-effect relations exists for each individual volunteer (data not shown). On day 8, the loop is flattened (data not shown). Furthermore, for a given plasma drug level, the degree of inhibition of the SBP response to Ang II seems to be higher on day 8 than on day 1. Fig 4B illustrates the same relation for the time-integrated data of individual subjects on days 1 and 8 taking into account the duration of the drug effect (the integral of the percentage of inhibition over 24 hours was related to the AUC of CV-11974 during the same period). This time-integrated dose-effect relation showed a linear pattern without any plateau, although the maximal inhibition is reached with the higher CV-11974 plasma concentrations. Although the magnitude of the inhibition of the blood pressure response to Ang II does not change, its duration is prolonged, leading to a linear increase of the time-integrated hemodynamic effect.

Neurohumoral Variables

The Table and Fig 5 depict PRA and Ang II plasma levels measured at the first and eighth administration of placebo or TCV-116. Both PRA and Ang II showed a marked dose-related increase 6 hours after drug intake, and this increase reached clearly higher values on day 8 than on day 1. Both variables had already increased significantly 4 hours after administration of 2, 4, and 8 mg TCV-116 on the first day ($P<.05$ versus placebo).

A very close correlation was found between PRA and plasma Ang II ($r=.91$, n=348, $P<.001$). There was also a negative correlation between the increase in PRA and the SBP response to exogenous Ang II ($r=-.456$, n=348, $P<.001$). Neither plasma norepinephrine nor plasma epinephrine (not shown) changed during the 8-day administration of TCV-116.

Fig 6 shows plasma aldosterone concentrations. Plasma aldosterone levels decreased after administration of single doses of TCV-116, but a similar decrease was also seen after placebo, reflecting the circadian variation in aldosterone concentrations.

Discussion

The present data demonstrate that TCV-116 is a potent, orally active Ang II antagonist with a relatively long duration of action. At peak effect (8 hours after drug intake), the 4-mg dose induced a 72±5% and 77±4% reduction in the systolic and diastolic blood pressure responses to Ang II, respectively. With the three higher doses used, ie, 2, 4, and 8 mg, a definite blocking effect was still present 24 hours after drug intake (day 1). With these three higher doses, the trough inhibition of Ang II pressor effect was greater on day 8 than on day 1, so that before drug intake on day 8, the pressor response to Ang II was only approximately 75% of the comparable response on day 1, suggesting that the drug exerts an effect that lasts for more than 24 hours. With repeated administration, no significant cumulative progressive enhancement of the blocking action could be observed at peak or trough. The dose dependency of the hemodynamic effect was clearly demonstrated with the four doses of TCV-116 throughout the treatment period (Fig 2).

The presence of constant trough levels from day 2 to day 8 suggests that no accumulation is occurring. These residual levels account for a negligible fraction (<6%) of the AUC values. The absence of a dose-proportional increase

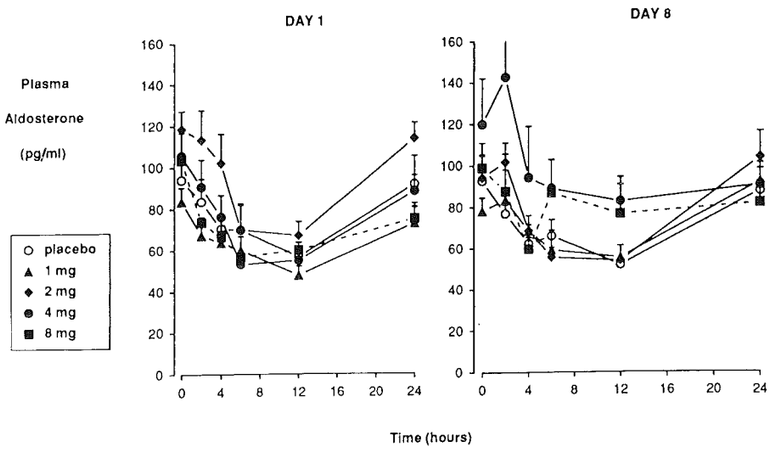

FIG 6. Line graphs show effect of 8 consecutive days of treatment with four oral doses of TCV-116 (1, 2, 4, and 8 mg/d) or placebo on plasma aldosterone levels on days 1 (left) and 8 (right).

in the AUC values between the 4- and 8-mg doses of TCV-116 could be due to nonlinear pharmacokinetics. Saturable absorption of TCV-116 or conversion to CV-11974 at a high dose is another possibility, as suggested by the prolongation of MRT values with increasing doses, that is also compatible with a lengthened absorption process. The CV-11974 concentration–blood pressure effect curve revealed an anticlockwise hysteresis loop, probably caused by pharmacokinetic influences determining the distribution of the drug to its site of action.

PRA and Ang II levels exhibited dose-related compensatory increases as described with previously tested Ang II antagonists.[6,34] The rise in renin and Ang I is similar to that observed after administration of ACE inhibitors.[35] With repeat administration of TCV-116, the increase was accentuated, the PRA and Ang II levels being much higher on day 8 than on day 1. The greater increase on day 8 than on day 1 in the PRA and Ang II values probably reflects the indirect effects of prolonged Ang II inhibition on sodium balance. The long duration of the blocking effect of TCV-116 is also reflected in the PRA and Ang II levels. Thus, 24 hours after the administration of 2, 4, or 8 mg, PRA and Ang II remained increased.

As expected, Ang II plasma levels were strongly correlated with PRA, and the response of both variables was inversely correlated with the SBP response to Ang II challenges. However, when individual data are considered, a substantial variability in these reactive increases becomes evident, as observed previously with losartan.[22] Therefore, the SBP response to Ang II challenges rather than the response of circulating levels of renin or Ang II should be used to predict the degree of Ang II receptor blockade in the individual subject.

Can such high Ang II plasma levels after an 8-day treatment with an Ang II antagonist finally reduce the effect of the Ang II antagonist? This is a priori unlikely to occur, because the exogenous Ang II probably induces much higher Ang II plasma levels than those occurring during the compensatory increase in renin secretion. Furthermore, the fact that for a given plasma CV-11974 level the degree of inhibition of the pressor response to Ang II tends to be greater on day 8 than on day 1 (Figs 2 and 4B) also suggests that the level of circulating Ang II has no measurable influence on the blocking effect of the AT_1 antagonist.

No effect of the Ang II antagonist on plasma aldosterone could be demonstrated. Indeed, plasma aldosterone levels decreased in treated subjects in a manner similar to that in the control group on placebo. This decrease in aldosterone levels is mainly due to the circadian rhythm of aldosterone secretion.[36]

The most relevant question clinically is how TCV-116 will compare as a therapeutic agent with the various agents currently available for blockade of the renin-angiotensin system. Obviously, results from the present study cannot provide any conclusive answer to this question because the drug was administered only to healthy volunteers. Nevertheless, it is already evident that this Ang II antagonist exhibits features that make it appear promising as a therapeutic agent. As with losartan,[18,19] it is orally active and does not seem to have any agonist effect. With the use of ACE inhibitors, the antihypertensive effect has been shown to be well correlated with the decrease in plasma Ang II concentration. Furthermore, blockade of the pressor effect of exogenous angiotensin has been shown to be strongly correlated with plasma Ang II[37] concentration. Consequently, since doses of 4 to 8 mg induce a more than 75% blockade of the pressor effect of Ang II in our study, we speculate that the oral antihypertensive dose of TCV-116 will be in the range of 4 mg/d. A similar projection was made with losartan. This agent was shown to maximally inhibit the pressor response to Ang II with doses less than 40 mg but less than or equal to 80 mg[18]; the full antihypertensive effect was subsequently obtained with 50 mg/d.[21,38-40]

In conclusion, TCV-116 appears to be a well-tolerated, orally active, potent, and long-lasting antagonist of Ang II in men. TCV-116 induced neurohumoral compensatory mechanisms that did not decrease its effect as measured by inhibition of blood pressure response to exogenous Ang II.

Acknowledgments

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