Effects of SC-52458, an Angiotensin AT$_1$ Receptor Antagonist, in the Dog
Ellen G. McMahon, Po-Chang Yang, Maribeth A. Babler, Osman D. Suleymanov, Maria A. Palomo, Gillian M. Olins, and Chyung S. Cook

We have previously reported on the basic pharmacologic properties of SC-52458 (5-[(3,5-dibutyl-1H-1,2,4-triazol-1-yl)methyl]-2-[2-(1H-tetrazol-5-ylphenyl)]pyridine), a novel angiotensin (AII) receptor antagonist that binds potently to AT$_1$ receptors in rat adrenal cortex and blocks AII-mediated contraction in isolated rabbit aorta. In the present study, the ability of SC-52458 to block AII pressor responses in conscious dogs was measured. In addition, we determined whether SC-52458 lowered mean arterial pressure compared to vehicle treatment although heart rate was not different in the two groups. The maximal pressor responses in conscious dogs was observed 24 h after dosing. SC-52458 measured by HPLC also were highest at the 2-h time point. After 24 h, the AII pressor response remained inhibited (by 35%) and SC-52458 was still measurable in plasma from treated dogs. In dogs made hypertensive by constriction of the left renal artery, SC-52458 lowered mean arterial pressure compared to vehicle treatment although heart rate was not different in the two groups. The maximal blood pressure lowering achieved with SC-52458 was similar to the maximal effect observed with the angiotensin converting enzyme inhibitor lisinopril. We conclude that SC-52458 blocks AII mediated pressor responses in normotensive, conscious dogs and SC-52458 is an efficacious antihypertensive agent in dogs with 2 kidney/1 clip renal hypertension.

Plasma concentrations of SC-52458 measured by HPLC also were highest at the 2-h time point. After 24 h, the AII pressor response remained inhibited (by 35%) and SC-52458 was still measurable in plasma from treated dogs. In dogs made hypertensive by constriction of the left renal artery, SC-52458 lowered mean arterial pressure compared to vehicle treatment although heart rate was not different in the two groups. The maximal blood pressure lowering achieved with SC-52458 was similar to the maximal effect observed with the angiotensin converting enzyme inhibitor lisinopril. We conclude that SC-52458 blocks AII mediated pressor responses in normotensive, conscious dogs and SC-52458 is an efficacious antihypertensive agent in dogs with 2 kidney/1 clip renal hypertension. © 1997 American Journal of Hypertension, Ltd. Am J Hypertens 1997;10:671–677

KEY WORDS: AT$_1$ receptor antagonist, angiotensin converting enzyme inhibitor, telemetry, renal hypertensive dog.

Inhibition of the renin-angiotensin system with converting enzyme inhibitors is an effective mode of treatment for essential hypertension and congestive heart failure.$^{2,3}$ The anti hypertensive mechanism underlying converting enzyme inhibition is presumably prevention of the production of angiotensin II (AII).$^4$ The conversion of angiotensinogen to angiotensin I (AI) by the enzyme renin is the rate limiting step in the production of AII and is the only known function of renin, whereas angiotensin converting enzyme (ACE) is known to act on a number of other biologically active peptides.$^5$ Specifically, it has been proposed that part of the antihypertensive activity of the ACE inhibitors might be due to potentiation of bradykinin effects.$^6$ Thus, blockade of angiotensin II receptors may lead to highly specific antihypertensive agents with an improved side effect profile compared to converting enzyme inhibitors. However, the efficacy of such agents could be reduced compared to ACE inhibitors. We have discovered a highly potent, selective, and orally available AT$_1$ receptor an-
tagonist that provides a useful tool for addressing this question in an established, renin-dependent form of hypertension, the 2 kidney/1 clip renal hypertensive dog. Although others have shown that acute administration of an AT\textsubscript{1} receptor antagonist (SK&F 108566) and an ACE inhibitor (enalapril) lower blood pressure comparably when administered acutely to renal hypertensive dogs, chronic dosing in this model has not been reported with either AT\textsubscript{1} receptor antagonists or ACE inhibitors. In addition, all previous studies have compared only single doses of AII receptor antagonists and ACE inhibitors that severely limits interpretation of these comparative data.

We have reported previously that SC-52458 is a potent, competitive, reversible antagonist of angiotensin II AT\textsubscript{1} type vascular receptors with a pA\textsubscript{2} of 8.18 in isolated rabbit aorta. Moreover, SC-52458 is a potent hypotensive agent when administered to hypertensive rats (spontaneously hypertensive and renal hypertensive) and sodium-deficient dogs. In the present study we used SC-52458 to test the hypothesis that chronic AII receptor blockade would be efficacious in a renin-dependent model of hypertension in the dog. Our results indicate that AII receptor blockade with SC-52458 is highly effective at lowering blood pressure in renal hypertensive dogs. This work has been reported previously in abstract form.

**METHODS**

**Angiotensin II Pressor Assay in Dog**  
*Animal Preparation* Female mongrel dogs (9 to 13 kg; Hazelton, Cumberland, VA) were anesthetized with isoflurane (Anaquest, Madison, WI) and were instrumented with a vascular access port (0.04 in. \times 0.09 in.) (Access Technologies, Skokie, IL) in the right carotid artery. Five days after recovery from the surgical procedure, the dogs were fasted overnight and the next morning the animals were placed in slings. The arterial port was connected to a Gould (Cleveland, OH) recorder for blood pressure monitoring via a Statham transducer (model P23ID, Oxnard, CA). An intravenous catheter was placed in the cephalic vein for administration of AII and blood sampling. After the blood pressure stabilized, an AII bolus (50 ng/kg intravenously; Sigma Chemical Co., St. Louis, MO) was given every 5 to 10 min until three similar pressor responses were obtained. The average of these three responses was considered 100% and all subsequent responses to AII were normalized to this response.

SC-52458 (Figure 1) was given by mouth in a gelatin capsule (30 mg/kg) whereas control animals received a capsule containing lactose (30 mg/kg). Blood samples (2.5 mL in EDTA) were taken at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after administration of SC-52458. All boluses were given at 0.5, 1, 2, 3, 4, 5, 6, 8, and 24 h after administration of SC-52458 or placebo. Blood samples were drawn just before administration of the AII boluses. After the 8-h time point, the dogs were disconnected from the blood pressure transducer, their intravenous catheters were removed, and they were returned to their home cages and allowed food and water ad libitum. The next morning the dogs were returned to the slings, the arterial line was reconnected, and a new intravenous catheter was inserted to allow for a 24-h blood sample and recording of the pressor response to injection of AII.

**Quantitative Analysis of SC-52458 in Plasma** Plasma concentrations of SC-52458 were determined using the assay procedure described previously. An aliquot (0.1 to 0.2 mL) of plasma sample was mixed with 0.1 mL of internal standard solution. The mixture was acidified with 0.1 N H\textsubscript{3}PO\textsubscript{4} to pH 3 and placed on a 1-mL CN Bond Elut column (Varian Harbor, CA) that was preconditioned with methanol, distilled water, and water acidified to pH 3. The column containing the sample was washed with 1 mL of water at pH 3 and 0.5 mL of a solvent mixture of methanol and water at pH 3 (20:80 vol/vol). SC-52458 and internal standard that were retained on the column were eluted with 0.3 mL of a mixture of acetonitrile and 0.1% triethylamine at pH 3 (1:1 vol/vol). An aliquot (75 \muL) of the eluate was injected onto an HPLC system.

HPLC was performed with a UV detector at a fixed wavelength of 234 nm and a NOVA PAK column (15 cm \times 3.9 mm, 4 \mum) with a RP-18 New Guard Cartridge (15 mm \times 3.2 mm, 7 \mum). The mobile phase was a mixture of 0.1% triethylamine in acidified water (pH 3) and acetonitrile (62.38 vol/vol). The flow rate of the mobile phase was 1 \mL/min. The sensitivity of the assay is 0.100 \mug SC-52458/mL. Acceptable precision and accuracy were obtained for concentrations above the sensitivity limit and within the standard curve range of 0.200 to 20.0 \mug SC-52458/mL of dog plasma.
Two Kidney/One Clip Renal Hypertensive Dog  

**Animal Preparation**  
Female beagle dogs (7 to 12 kg) were obtained from Hazelton, (Cumberland, VA). Under sterile conditions, the animals were anesthetized with isoflurane (Anaquest Inc., Madison, WI) and a catheter connected to a radiotelemetry blood pressure transmitter (Mini-Mitter Co., Inc., Sunriver, OR) was implanted in the left femoral artery. The transmitter capsule was anchored subcutaneously to the abdominal region and the incision was closed. After recovery from the anesthesia, the animals were returned to their home cages. A receiver (Data Sciences, Inc., St. Paul, MN) under the dog’s cage was used to collect mean arterial pressure (MAP) and heart rate (HR) values every 5 min. A Compaq 486 computer (Compaq Computer Corp., Houston, TX) was used to process the data using DataQuest IV 2.0 (Data Sciences, Inc., St. Paul, MN). Six- or 24-h averages were calculated using this system.

Approximately 1 week after telemetry unit implantation, the animals were anesthetized with isoflurane and under sterile conditions, a left flank incision was made. An electromagnetic flowmeter (Carolina Medical Electronics, Inc., King, NC) was placed around the left renal artery to record blood flow. A 3-mm stainless steel Goldblatt clamp (Keystone Automation Technology, Emporium, PA) was then placed around the left renal artery and tightened to reduce blood flow by 70% to 80%. The clamp was tightened until the reduction in flow was maintained for at least 15 min. The screw of the clamp was then secured with cyanoacrylate glue and the flank incision was closed. Before clamping, a 3-mL blood sample was obtained and 4 days after constriction of the left renal artery, a second 3-mL sample was obtained for measurement of plasma renin activity (PRA) (see Method section below). Dogs with mean arterial pressure that increased less than 25 mm Hg on day 5 after renal artery constriction were removed from further study. Using these criteria, three dogs were eliminated from the study. SC-52458 was dissolved in 1 N NaOH and then 0.1 N HCl was added slowly to adjust the pH to approximately 9.5. Distilled water was added to a final concentration of 75 mg/mL. The compound was administered at 10, 30, or 60 mg/kg loaded into a gelatin capsule daily at 12:30 PM for 4 days, beginning 6 days after renal artery constriction. Vehicle-treated animals received capsules containing distilled water adjusted to pH 9.5 with 1 N NaOH. In separate experiments, lisinopril (U.S.P.C., Rockville, MD) was administered in a gelatin capsule to renal hypertensive dogs at 12:30 PM for 4 days, beginning 6 days after renal artery constriction.

**Plasma Renin Activity**  
A 3-mL blood sample was collected in EDTA-containing Vacutainer tubes (Becton Dickinson, Rutherford, NJ) and the blood was centrifuged at 4000 g for 10 min at 4°C to separate plasma from packed cells. Plasma samples (200 µL) were analyzed for plasma renin activity (PRA) using the method of Sealey and Laragh with modifications. The 200-µL plasma samples were incubated with 3 µL of 0.5 mol/L 8-hydroxyquinoline (Aldrich Chemical Co., Milwaukee, WI), 3 µL of 10% neomycin sulfate (Sigma), 1 µL of phenylmethylsulfonylfluoride (Calbiochem, La Jolla, CA), and 20 µL of 0.5 TES buffer (Sigma) at pH 7.4 at 37°C for 90 min. The reaction was terminated by placing the mixture tubes in an ice bath. The amount of AI generated was determined by an AI radioimmunoassay (Dupont-NEN, Boston, MA) and was expressed as nanograms of angiotensin I per milliliter of plasma per hour.

**Statistical Analysis**  
All statistical calculations were performed using SAS (SAS Institute, Cary, NC). A mean comparison resulting in a P value of .05 or less was considered statistically significant. For inhibition of the AII pressor response, a two-way (treatment group by day), repeated measures (on time point) ANOVA was performed. The means at each time point were tested for significant differences from 100% control (same as initial values). For the plasma levels of SC-52458, a one-way, repeated measures ANOVA was performed to compare the means across time points.

For the renal hypertensive dog study, comparison of the PRA and MAP for the SC-52458 group and the lisinopril group was performed using a one-way ANOVA. The analysis was conducted both on the post-clip values and on the change from pre-clip to post-clip values. The analysis on the change variable also allowed tests of significant difference from pre- to post-clip for each group by testing for significant differences from zero.

Because of the circadian pattern of HR and blood pressure (values highest at mid-morning and then decreasing during the evening and at night), daily averages of these parameters were computed and compared. The analysis was performed on the change in HR and blood pressure from day 0 to days 1, 2, 3, and 4. PROC MIXED in SAS was used to perform the two-way (treatment group by day), repeated measures ANOVA. The t test means comparisons were made to the vehicle group. Means averaged over all 4 days were compared if the analysis found no significant difference in the pattern of mean differences over days (P value for interaction >.10).

**RESULTS**

**Angiotensin II Pressor Assay in Dog**  
The AII pressor response was inhibited by approximately 60% within 1
sensitive analysis, the ANOVA calculations were conducted on the change in MAP and HR for each dog from day 0.

As shown in Figure 4A, SC-52458 lowered MAP each day in a dose-dependent manner compared to the vehicle. The pattern of differences between means was not significantly different over days (interaction \( P \) value > .10). The drop in MAP was consistent over time, averaging 4.0, 11.9, and 16.4 mm Hg in the SC-52458 groups at 10, 30, and 60 mg/kg, respectively, compared to 3.3 mm Hg in the vehicle group (all means averaged over the 4 treatment days). These reductions in MAP were significantly different from the vehicle mean at the 30- and 60-mg/kg doses of SC-52458. For SC-52458 at 30 and 60 mg/kg, the mean change in MAP was significantly less than zero on each of the treatment days, indicating sustained blood pressure lowering effects over the 4 days. For the vehicle, the mean change in MAP was significantly less than zero on the last day of the study. This drift of blood pressure slightly downward over time is a consistent feature of this model. Lisinopril-treated animals demonstrated a significant decrease in blood pressure on each of the treatment days compared to the control response.

Plasma concentrations of SC-52458 were significantly higher at 2 h after SC-52458 administration (9.74 ± 1.24 \( \mu g/mL \)) than at any other time point. SC-52458 was still measurable in plasma 24 h after dosing (0.29 ± 0.09 \( \mu g/mL \)) (Figure 3). The Spearman correlation coefficient indicated a statistically significant negative relationship between the plasma level and the AII pressor response (\( rs = 0.93, P = 0.0025 \)).

Two Kidney/One Clip Renal Hypertensive Dog

There were no significant differences between the six treatment groups in PRA and MAP post-clip values or in the amount of change from pre-clip to post-clip values (Table 1). In all cases, the post-clip values were significantly elevated.

Figures 4 and 5 show the 6-h means for each treatment group. Because of the obvious circadian pattern of MAP and HR in the study, daily averages were computed for 5 days (0, 1, 2, 3, and 4). To obtain a more

FIGURE 2. Effect of oral administration of SC-52458 on the pressor response to AII (50 ng/kg, intravenously). Dogs were dosed with SC-52458 at 30 mg/kg or placebo at time 0. Control pressor responses were 37 ± 6 mm Hg and 44 ± 6 mm Hg in placebo and SC-52458-treated dogs, respectively. Symbols represent mean values ± SEM, \( n = 4 \) for each group. * indicates statistically significant inhibition of the AII pressor response compared to the control response.

FIGURE 3. Plasma concentrations of SC-52458 in conscious dogs after administration of SC-52458 at 30 mg/kg, orally at time 0. Symbols represent mean values ± SEM, \( n = 4 \) for each group. The correlation between plasma level of SC-52458 and inhibition of the AII pressor response was statistically significant (\( rs = 0.93, P = .025 \)).
TABLE 1. PLASMA RENIN ACTIVITY AND MEAN ARTERIAL PRESSURE IN DOGS BEFORE AND AFTER LEFT RENAL ARTERY CONSTRICTION AND BEFORE VEHICLE OR DRUG TREATMENT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Plasma Renin Activity (ng Al/mL/h)</th>
<th>Mean Arterial Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Precip Day 4</td>
<td>Postclip Day 4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>—</td>
<td>1.91 ± 0.67</td>
<td>13.11 ± 2.88*</td>
</tr>
<tr>
<td>SC-52458</td>
<td>10 mg/kg</td>
<td>0.95 ± 0.26</td>
<td>11.71 ± 3.46*</td>
</tr>
<tr>
<td>SC-52458</td>
<td>30 mg/kg</td>
<td>1.23 ± 0.52</td>
<td>7.87 ± 1.80*</td>
</tr>
<tr>
<td>SC-52458</td>
<td>60 mg/kg</td>
<td>1.24 ± 0.14</td>
<td>5.79 ± 1.22*</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>1 mg/kg</td>
<td>2.30 ± 1.07</td>
<td>9.50 ± 0.87*</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>3 mg/kg</td>
<td>1.6 ± 0.58</td>
<td>12.50 ± 1.45*</td>
</tr>
</tbody>
</table>

Mean values ± SEM, n = 6 for each group, except SC-52458 at 10 mg/kg where n = 5 and lisinopril where n = 4.
*P < .05 compared to preclip values.

ficient from the means for lisinopril at 1 mg/kg and for SC-52458 at 30 mg/kg, but was not statistically different from SC-52458 at 60 mg/kg.

As shown in Figures 4B and 5B, the circadian pattern of HR was more prominent than the circadian variation in blood pressure, with heart rates highest at mid-morning, decreasing during the rest of the day, and reaching the lowest value at night. The pattern of differences between means was not significantly different over days (interaction P value >.10). When averaged over the 4-day treatment period, there were no statistically significant differences between any of the treatment group means and the mean for the vehicle group. Therefore, both SC-52458 and lisinopril decreased MAP in a significant and similar manner with no evidence of reflex tachycardia when compared to the vehicle-treated animals.

DISCUSSION

Although ACE inhibitors are highly effective antihypertensive agents in most patients, these compounds can cause serious side effects, namely cough and angioedema, in some patients.11 The mechanism of these side effects is believed to be potentiation of bradykinin effects by inhibition of ACE (also known as kininase II), the enzyme responsible for the degradation of bradykinin. In addition, numerous reports have now demonstrated that AII can be formed, at least in human heart, by a chymotrypsin-like enzyme or chymase that is not blocked by ACE inhibitors.12 Thus, AT1 receptor blockers may have an improved side effect profile and could be more efficacious during long-term use than ACE inhibitors. Preliminary data with the first AT1 receptor antagonist available for human use, losartan, indicates that these compounds may offer therapeutic advantages over existing therapies.13

As demonstrated in this study, SC-52458 is an effective blocker of the AII pressor response in conscious, normotensive dogs dosed with the compound at 30 mg/kg. Blockade of the pressor response to an AII challenge correlated well with the quantity of SC-52458 measured in plasma. Even 24 h after a single dose of SC-52458, the pressor response to AII was still inhibited by 35%. These properties of SC-52458 suggested to us that SC-52458 might be an effective antihypertensive agent when dosed chronically in a hypertensive animal model and therefore, we studied SC-52458 in the 2 kidney/1 clip renal hypertensive dog.

Renal artery constriction in dogs results in a renin-dependent hypertension, although the renin dependency of the hypertension is transient and often variable.7,14 We chose to test inhibitors of the renin-angiotensin system in this model from 6 to 10 days after constriction of the renal artery, a period of time when both PRA and MAP are substantially elevated and reasonably stable.15 In the present study, a telemetry device was used to monitor the blood pressure and HR continuously at 5-min intervals throughout the dosing interval with the animals freely moving in their home cages. Observations from the vehicle-treated group indicate that a prominent circadian pattern of blood pressure variation is present in the 2 kidney/1 clip hypertensive dogs, which is similar to the profile in hypertensive patients.16 Blood pressure was highest in the morning and reached its nadir each night. Both SC-52458 and lisinopril produced a significant reduction in MAP in the hypertensive dogs and the same maximal decrease was observed with both compounds. Previous data from our laboratory indicated that 3 mg/kg of lisinopril was a maximal dose for blood pressure lowering in the salt-depleted dog (unpublished observations). Therefore, these data suggest that the blood pressure reduction in this model is most likely attributable to inhibition of the
renin-angiotensin system and not other effects of the ACE inhibitors such as bradykinin potentiation. This conclusion is supported by the fact that renin inhibitors produce a similar maximal decrease in blood pressure in this model.

As reported previously for renin inhibitors and ACE inhibitors in salt-depleted and renal hypertensive animals, SC-52458 and lisinopril lowered MAP effectively in the 2 kidney/1 clip hypertensive dog without any significant reflex tachycardia. Our study is particularly powerful in confirming these previous observations, as our measurements were obtained in dogs freely moving in their home cages using a radio-

FIGURE 4. Effect of oral administration of SC-52458 and vehicle on (A) mean arterial pressure (MAP) and (B) heart rate in 2 kidney/1 clip renal hypertensive dogs with implanted radiotelemetry transmitters for continuous measurement of blood pressure and heart rate. Dosing was initiated 6 days after renal artery constriction. Measurements were made at 5-min intervals and 6-h averages were calculated. Symbols represent mean values ± SEM. n = 6 for all groups except SC-52458 at 10 mg/kg where n = 5.

telemetric method rather than under conditions of stressful restraint. This lack of reflex tachycardia associated with inhibition of the renin-angiotensin system is believed to be due to removal of an AII potentiation of baroreflexes, although no conclusive evidence is available in support of this hypothesis. However, this lack of reflex tachycardia is a clear advantage of using renin-angiotensin system inhibitors rather than other antihypertensive agents that tend to increase HR in response to a decrease in blood pressure.

In conclusion, SC-52458 is a potent, effective blocker of the AII pressor response in the dog model and is equally efficacious to the ACE inhibitor lisinopril in

FIGURE 5. Effect of oral administration of lisinopril and vehicle on (A) mean arterial pressure (MAP) and (B) heart rate in 2 kidney/1 clip renal hypertensive dogs with implanted radiotelemetry transmitters for continuous measurement of blood pressure and heart rate. Dosing was initiated 6 days after renal artery constriction. Measurements were made at 5-min intervals and 6-h averages were calculated. Symbols represent mean values ± SEM. n = 6 for vehicle group, n = 4 for lisinopril at 1 and 3 mg/kg.
2 kidney / 1 clip renal hypertensive dogs dosed chronically over 4 days. In conscious dogs monitored in their home cages by radiotelemetry, no significant changes in HR occurred in response to significant decreases in blood pressure with either the ACE inhibitor lisinopril or the AT₁ receptor antagonist SC-52458. SC-52458 is likely to be a potent and effective antihypertensive agent in humans, although clinical testing of the compound is obviously required to confirm this.

ACKNOWLEDGMENTS

We thank Stephen Bittner, Michael Baratta, Larry Kosobud, and Krys Miller for excellent technical assistance and Jeanne Sebaugh for her assistance in statistical analyses of the data.

REFERENCES