Pharmacology of a Phosphorus-Containing Novel Angiotensin Converting Enzyme Inhibitor, SQ 29 852 in Anesthetized Dogs

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The effects of (S)-1-[6-amino-2[[hydroxy(4-phenylbutyl)phosphinyloxy]-1-o xoetyl]-l-proline (SQ 29 852), a phosphorus-containing novel angiotensin converting enzyme inhibitor (ACEI), which is synthesized aiming an ACEI with long-lasting activity and with few side effects, were studied using anesthetized dogs. SQ 29 852 was equipotent with captopril to modify blood pressure response of the animals to angiotensin I (Ang I) and bradykinin (Bdk). An intravenous infusion of SQ 29 852 at 0.1 mg/kg/min for 30 min caused a remarkable hypotension without reflex tachycardia in open-chest dogs. In these animals cardiac contractility (dP/dt max of left ventricular pressure) appeared to be reduced by SQ 29 852 without any changes in right atrial pressure (RAP), left ventricular end-diastolic pressure (LVEDP) and aortic blood flow (AoF, cardiac output). In sodium-restricted dogs, the hypotension and renal vasodilation by SQ 29 852 (at 0.01, 0.1, and 1 mg/kg, i.v.) were slightly pronounced compared with animals fed with normal diet. It is demonstrated from these results that SQ 29 852 has comparable potency with captopril to inhibit angiotensin converting enzyme (ACE) activity and as a common a pharmacological profile as ACEI. SQ 29 852 may be a favorable antihypertensive agent, if its long-lasting activity and few side effects are confirmed.

Keywords — (S)-1-[6-amino-2[[hydroxy(4-phenylbutyl)phosphinyloxy]-1-oxoetyl]-l-proline (SQ 29 852); angiotensin converting enzyme inhibitor (ACEI); anesthetized dog

Introduction

Recently a new series of phosphorus-containing angiotensin converting enzyme inhibitors (ACEIs) has been synthesized aiming new compounds with longer-lasting hypotensive activity and fewer side effects compared with previously developed ACEIs.1-4 The novel molecules possess a hydroxyphosphinyl function as a binding site to the active moiety of the angiotensin converting enzyme. Among them SQ 29 8525 (Fig. 1) and SQ 27 5195 have been reported to have successful ACEI activity. For a phosphinic acid, SQ 27 519, however, an esterification into a prodrug form (fosinopril) is necessary because of its poor oral activity.2,5 In contrast, it has been reported that SQ 29 852, a phosphonate-containing ACEI can directly reveal a potent ACE inhibiting activity by oral application and its duration is longer than that of captopril.1)

The pharmacological profiles of phosphorus-containing ACEIs in vivo have not yet been studied in detail, whereas the potencies in inhibition of ACE have been mentioned.1-4) Thus, in the present study we examined acute effects of a representative phosphorus-containing ACEI, SQ 29 852 in anesthetized dogs. Namely, the following items were pharmacologically examined:

1. The inhibitory effect of SQ 29 852 on ACE was compared with that of captopril focusing on the attenuation of the response to angiotensin (Ang I) and the augmentation of that to bradykinin (Bdk).
2. Cardiovascular effects of SQ 29 852 were fol-

Fig. 1. Molecular Structure of SQ 29 852

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allowed in open-chest dogs.
3. Hypotensive and peripheral vasodilating effects of SQ 29 852 were compared using dogs fed with a normal, and with a low-sodium diet.

Materials and Methods

A total of 42 mongrel random-source dogs of either sex weighing 8.2—18.5 kg were used for this study. The animals were fed a daily ration of CD-5 canine diet (Clea Japan, Tokyo) and water was provided ad libitum, unless otherwise stated.

Comparison of Blood Pressure Response to Ang I or Bdk before and after Intravenous Injection of SQ 29 852 and Captopril — The animals were anesthetized with 30 mg sodium pentobarbital/kg, i.v. Supplementary sodium pentobarbital was injected as required through a rubber tube placed in the femoral vein. Ang I (1 μg/kg) and Bdk (0.01 μg/kg) were administered intravenously and the maximum blood pressure responses to these peptides were compared before and 5 min after intravenous injection of SQ 29 852 or captopril (at doses of 0.01—10 mg/kg). The potencies of ACEIs to inhibit Ang I-response and to potentiate Bdk-response were compared using IC50 and EC50 values, respectively. The IC50 and EC50 values were defined as follows:

IC50, the intravenous dose of ACEIs which attenuates the maximum blood pressure response to Ang I (1 μg/kg, i.v.) by 50%

EC50, the intravenous dose of ACEIs which augments the maximum blood pressure response to Bdk (0.01 μg/kg, i.v.) by 50%

Investigation of Hemodynamic Effects of SQ 29 852 — The animals were intubated under pentobarbital anesthesia (30 mg/kg, i.v.) using a cuffed endotracheal tube, and respired by the use of a Harvard 607 respirator (Millis, Massachusetts) at 20 strokes/min with a tidal volume setting of 20 ml/kg. Then the heart was exposed via a median thoracotomy, and suspended in a pericardial cradle. A non-cannulating type probe of an electromagnetic flow meter was attached around the ascending aorta for measuring aortic blood flow (AoF). A polyethylene tube was inserted into the left ventricle to measure left ventricular end-diastolic pressure (LVEDP). The left ventricular pressure was differentiated and the maximal rate of the changes in the pressure dP/dtmax (cardiac contractility) was monitored. A rubber tube was placed in the right atrium for measuring right atrial pressure (RAP). Blood pressure and heart rate were also monitored by the same procedure as that stated in the preceding paragraph.

SQ 29 852 (0.1 mg/kg/min) was infused intravenously for 30 min through a rubber tube placed in the femoral vein, since the infusion was better to obtain consistently steady states of the hemodynamic parameters than a bolus injection. The above mentioned parameters were observed during the infusion, and at least for 1 h after the cessation of dosing.

Measurement of Peripheral Blood Flow in Dogs with or without Activated Renin-Angiotensin-Aldosterone System (RAAS) — For 7—10 d prior to this series of experiments, each 10 animals were fed a daily ration of 300 g of either CD-5 canine diet (Clea Japan, Tokyo) containing 440 mg sodium/100 g or a low sodium diet (Clea Japan) containing 164.5 mg sodium/100 g. After this controlled feeding, peripheral venous blood of the animals was taken under conscious condition to determine plasma renin activity (PRA) using Renin RIA beads® (Dinabot): Thereafter, the animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and the femoral or the left renal artery was exposed. A non-cannulating type probe of an electromagnetic flow meter was attached around the artery to measure the blood flow. Blood pressure and heart rate were also monitored by the same procedure as those previously stated. Then SQ 29 852 (0.01, 0.1, and 1 mg/kg, i.v.) was injected in a dose-increasing manner at an interval longer than 1 h.

Changes in the femoral blood flow (FBF) were compared in two groups of 5 animals for each fed with a normal, or with a low-sodium diet, respectively. Changes in the renal blood flow (RBF) were also compared similarly between 5-animal groups given a normal and a low-sodium diet. Peripheral resistance at each vas-
circular bed was calculated by dividing the mean blood pressure by the blood flow rate.

Apparatuses for the Measurement and Recording — Blood pressure and LVEDP were measured by means of a pressure transducer (P23Db, Statham, Hato Rey) and a pressure-force displacement amplifier (1206B or 1236, San-ei, Tokyo). Heart rate was determined from the systemic blood pressure trace by a tachometer (T-149, Data graph, Tokyo). \( \frac{dP}{dt_{\text{max}}} \) was calculated from changes in the left ventricular pressure by a differentiator (DF-1, Data graph, Tokyo). The aortic, femoral, and renal blood flow were measured using an electromagnetic flow meter (T-500, Narco, Houston) and a direct current amplifier (1103, San-ei, Tokyo). These parameters were recorded on a rectigraph (Recti-Horiz-8K, NEC-San-ei, Tokyo).

Chemicals — The following drugs were used in the present study: angiotensin I (Ang I) and bradykinin (Bdk) (Peptide Institute, Osaka), pentobarbital sodium (Tokyo Kasei, Tokyo), SQ 29 852 ((S)-1[6-amino-2[[hydroxy(4-phenylbuyl]-phosphinyl]oxy]-1-oxohexyl]-L-proline) and captopril (Squibb Japan, Tokyo).

Statistics — Values in the present study are represented as means ± S.E.M. Statistical significance of differences between time-matched values of two groups in the measurement of cardiovascular parameters of dogs were established by Mann-Whitney U-test. Differences between pre- and post-injection values were established by Wilcoxon’s signed-rank test. All the other determination of statistical significance between two group means were determined by single-factor analysis of variance followed by Student’s \( t \)-test. In all cases differences are considered significant at \( p < 0.05 \).

Results

Modification of Blood Pressure Responses to Ang I and Bdk

SQ 29 852 attenuated Ang I-induced increase, and augmented Bdk-induced decrease of blood pressure in a dose-dependent manner. The potencies of SQ 29 852 both in the attenuation and the augmentation of respective responses to Ang I and Bdk were similar to those of captopril (Fig. 2). \( ID_{50} \) values of Ang I-response were

![Diagram](image)

Fig. 2. Modification by SQ 29 852 or Captopril of Blood Pressure Responses to Angiotensin I and Bradykinin in Anesthetized Dogs

(A) The attenuation of the hypertension by angiotensin I (Ang I) at 1 \( \mu g/kg \), i.v. and (B) the augmentation of the hypotension by bradykinin (Bdk) at 0.01 \( \mu g/kg \), i.v. with or without pre-injection of SQ 29 852 (solid symbols) and captopril (open symbols) at 0.0–1 \( \mu g/kg \), i.v. for both ACEIs.

The symbols indicate the mean values of 10 animals. Vertical bars represent S.E.M.
TABLE I. Effects of Intravenous Infusion of SQ 29 852 on Hemodynamics in Anesthetized Dogs

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>During infusion (0.1 mg/kg/min, for 30 min)</th>
<th>Recovery after the cessation of infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>135.8±8.5</td>
<td>119.3±9.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121.4±9.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75.9±6.0</td>
<td>62.7±6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.8±5.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>96.9±6.6</td>
<td>80.1±7.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.0±6.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>150.1±4.8</td>
<td>149.4±5.5</td>
<td>152.4±6.4</td>
</tr>
<tr>
<td>AoF (ml/min/10 kg)</td>
<td>782±63</td>
<td>785±70</td>
<td>823±89</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td>2799±192</td>
<td>2501±222&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2612±240</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.8±1.1</td>
<td>4.5±1.5</td>
<td>3.5±1.0</td>
</tr>
<tr>
<td>RAP (cmH₂O)</td>
<td>3.3±0.4</td>
<td>3.4±0.4</td>
<td>3.5±0.4</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. of 9 animals. <sup>a</sup> p<0.05, <sup>b</sup> p<0.01, significantly different from the values before starting infusion (Wilcoxon's signed-rank test).

SBP, systemic blood pressure; HR, heart rate; AoF, aortic blood flow (cardiac output); dP/dt<sub>max</sub>, maximal rate of rise in the left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; RAP, right atrial pressure.

1.0±0.40 mg/kg (2.3±0.92 µmol/kg) (N=5) for SQ 29 852 and 0.38±0.16 mg/kg (1.7±0.74 µmol/kg) (N=5) for captopril (p=0.651), and

ED<sub>50</sub> values of Bdk-response were 0.037±0.011 mg/kg (0.086±0.031 µmol/kg) (N=5) for SQ 29 852 and 0.026±0.013 mg/kg (0.12±0.061

Fig. 3. Effects of Intravenously Administered SQ 29 852 on Blood Pressure and Heart Rate, in the Dogs Fed with Normal and Low-Sodium Diet

SQ 29 852 (0.01, 0.1 and 1 mg/kg) was injected in a dose-increasing manner, and changes in mean blood pressure (MBP) and heart rate (HR) were compared between normal (open symbols) and sodium-restricted groups (solid symbols). Symbols with vertical bars represent the mean values with S.E.M. of 5 animals.

<sup>a</sup> p<0.05, <sup>b</sup> p<0.01, significantly different from the control values (Wilcoxon's signed-rank test).
μmol/kg) (N=5) for captopril (p=0.617), respectively.

**Changes in Hemodynamics by SQ 29 852**

In these experiments SQ 29 852 was intravenously infused, since it was necessary for successful measurements of dP/dt$_{\text{max}}$ and LVEDP. SQ 29 852 was at first infused for 30 min at 0.01, 0.1, and 1 mg/kg/min, and the effects of these doses were compared in order to find an optimal dose to examine the hemodynamic effects. At 0.01 mg/kg/min, the agent did not cause marked hemodynamic changes except a slight hypotension. On the other hand, at 0.1 and 1 mg/kg/min, it caused a remarkable decrease in blood pressure, and the hypotensive potencies at both doses were comparable. Namely the hypotensive effect of SQ 29 852 was likely to be saturated at doses around 0.1 mg/kg/min. Thus, we decided to examine the hemodynamic effects of SQ 29 852 at 0.1 mg/kg/min.

The results are shown in Table I. Blood pressure was decreased markedly by intravenous infusion of SQ 29 852 at 0.1 mg/kg/min for 30 min. This hypotension continued at least for 1 h after the withdrawal of infusion. In parallel with the decrease in blood pressure, dP/dt$_{\text{max}}$ tended to decrease, while heart rate, LVEDP, AoF, and RAP did not change.

**Changes in Peripheral Blood Flow and Resistance**

The sodium-restricted dogs had higher PRA (4.8 ± 0.8 ng Ang I/ml/h, N=10) than the animals fed with normal diet (2.8 ± 0.4 ng Ang I/ml/h, N=10, p<0.05). SQ 29 852 similarly decreased blood pressure in the animals of both groups (Fig. 3), and the hypotension in the sodium-restricted group tended to be pronounced compared with the group fed with normal diet. In both groups heart rate did not obviously change after the injection of SQ 29 852 (Fig. 3).

Neither FBF nor the femoral vascular

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**Fig. 4. Effects of Intravenously Administered SQ 29 852 on Blood Flow and Peripheral Vascular Resistance in the Hind Limb of the Dogs Fed with Normal and Low-Sodium Diet**

SQ 29 852 (0.01, 0.1 and 1 mg/kg) was injected in a dose-increasing manner, and changes in femoral blood flow (FBF) and the peripheral resistance (PR) were compared between normal (open symbols) and sodium-restricted groups (solid symbols).

Symbols with vertical bars represent the mean values with S.E.M. of 5 animals.

a) p<0.05, significantly different from the control values (Wilcoxon's signed-rank test). b) p<0.05, c) p<0.01, significantly different from the values in the normal group (Mann-Whitney U-test).
Resistance in the dogs fed with normal diet was markedly changed by SQ 29 852 (Fig. 4). In contrast, a marked and dose-related decrease of FBF which was accompanied by an increase in the femoral vascular resistance was observed in the sodium-restricted animals (Fig. 4). On the other hand, RBF was increased, and the renal vascular resistance was markedly reduced by SQ 29 852 especially at 0.1 and 1 mg/kg in the animals of both groups. The reduction of renal vascular resistance in sodium-restricted animals was somewhat pronounced compared with the animals fed with normal diet when 1 mg/kg of SQ 29 8562 was administered (Fig. 5).

**Discussion**

According to the reports by Cushman et al.,4,5 SQ 29 852 is less potent than captopril for inhibition of ACE activity in rat plasma and tissue homogenates. In the present study using dogs, however, the modifications of the responses to Ang I and Bdk by SQ 29 852 were comparable with those by captopril. Therefore it is indicated in anesthetized dogs that intravenously administered SQ 29 852 has an equipotent ACE inhibiting activity to captopril.

The experiments in open-chest dogs were performed to clarify the hemodynamic effects of SQ 29 852. Here AoF, LVEDP, and \( \frac{dP}{dt_{\text{max}}} \) were measured in addition to blood pressure and heart rate. Blood pressure decreased immediately, and this hypotension came up to a plateau within 10 min after starting the infusion of SQ 29 852. \( \frac{dP}{dt_{\text{max}}} \) tended to be decreased by the agent. However, this could not lead to any cardiac depression, since it was not accompanied by any marked changes in the other parameters except
blood pressure. This apparent decrease in cardiac contractility may be due to an indirect reduction of sympathetic nervous tone. Actually it has been reported that Ang II is concerned with the facilitation or the maintenance of the sympathetic nervous tone, and that ACEI inhibits sympathetic excitation by decreasing the level of endogenous Ang II. Similarly the lack of reflex tachycardia during the SQ 29 852-induced hypotension may be the result of the offset by the reduction of sympathetic tone against the reflex. A similar finding, which can also be referred to the decrease of endogenous Ang II level and the apparent sympathetic inhibition, has been reported on the effects of captopril.

Sodium-depletion or sodium-restriction is a well-grounded procedure, to study the regulation by stimulated RAAS of blood pressure, and to evaluate the hypertensive effect of ACEIs being based on RAAS in vivo, though ACEI-induced acute hypotension is not always related to the inhibition of RAAS but to an increase in the level of endogenous kinins, prostaglandins or other parameters. Thus we compared the hypotension caused by SQ 29 852 in the sodium-restricted dogs with that in the animals fed with a normal diet, and found that the sodium-restricted animals tended to be more sensitive in response to SQ 29 852 than the animals given a normal diet. Such a finding is similar to that in captopril, and it is, therefore, concluded that at least in part, RAAS seems to regulate the acute blood pressure response to SQ 29 852.

In the dogs fed with a normal diet, FBF did not change even when the hypotension was observed. In contrast, FBF decreased and the femoral vascular resistance increased with the hypotension in the sodium-restricted animals. A possible decrease in circulating blood volume by low-sodium intake, the changes of blood distribution, and the relatively pronounced hypotension in the sodium-restricted animals might directly affect the FBF and femoral vascular resistance. On the other hand, RBF increased in the animals of both groups. In the sodium-restricted animals, the duration of the decrease in renal vascular resistance by 1 mg/kg of SQ 29 852 was somewhat longer than that in the animals fed a normal diet. These results suggest that the regulation of renal vascular tone should be more closely related to RAAS than in the femoral vasculature.

The content of sodium in the low-sodium diet used in the present study was somewhat higher than those used as a sodium-deficient diet in previous reports. However, sodium-restricted dogs in the present study had significantly higher PRA than animals fed with a normal diet, and was comparable with those in previously reported sodium-deficient dogs. Therefore our procedure to obtain a sodium-restricted condition was thought to be enough to stimulate RAAS.

In summary, the data from the present study using anesthetized dogs demonstrate that intravenously administered SQ 29 852 has a potent ACE inhibiting efficacy which is comparable with captopril, though SQ 29 852 has been reported to be less potent than captopril in inhibiting ACE activity in rats. It is also confirmed that, like captopril, SQ 29 852 decreased blood pressure without reflex tachycardia and its hypertensive activity tended to be pronounced in a sodium-restricted condition with activated RAAS. Although there are no comparative studies on the antihypertensive efficacy of SQ 29 852 and other ACEIs, it has been reported that ACE-inhibiting duration of SQ 29 852 is similar to that of enalapril. Thus, SQ 29 852, a novel phosphorus-containing ACEI may be a favorable ACEI which does not need prodrug form for exhibiting its activity, if its long-lasting hypertensive activity and few side effects are confirmed.

References


