Effects of the Oral Converting Enzyme Inhibitor, SQ 14225, in a Model of Low Cardiac Output in Dogs

RONALD H. FREEMAN, JAMES O. DAVIS, GARY M. WILLIAMS, JACK M. DEFORREST, ANDREA A. SEYMOUR, AND BRIAN P. ROWE

SUMMARY  Dogs with thoracic caval constriction retain sodium and develop ascites and edema. The role of the renin-angiotensin-aldosterone system in this model of low output failure was evaluated before, during, and after administration of the new orally active converting enzyme inhibitor, 2-D-methyl-3-mercaptopropanoyl-L-proline (SQ 14225). The acute response to the initial oral dose of SQ 14225 (10 mg/kg) consisted of a striking fall in plasma aldosterone concentration (PAC) from 22.7 and 29.9 ng% to 10.7, 11.9, and 11.0 ng% (P < 0.05) after 67.5, 112.5, and 157.5 minutes; sodium excretion increased from 1.9 and 1.9 μEq/min to 19.9, 22.4, and 17.8 μEq/min. Arterial pressure and filtration fraction decreased (P< 0.05), and plasma renin activity (PRA) increased (P < 0.05) after the initial dose of SQ 14225; clearance of para-aminohippuric acid (PAH) and creatinine did not change significantly. The daily responses for 3-4 days to SQ 14225 (35 mg/kg per day, given as doses of 10, 10, and 15 mg/kg) were a decrease in PAC from 50 ± 15 and 32 ± 10 ng% to 10 ± 4 ng% on the 4th day, a value not statistically different from normal (P > 0.05), and an increase in sodium excretion from 2.9 to 2.0 mEq/day to 5.7, 10.0, 12.4, and 32.9 mEq/day on a sodium intake of 35 mEq/day (P < 0.05 for the last 2 days). Arterial pressure and creatinine clearance decreased (P < 0.05). PRA increased transiently on day 1 of SQ 14225 and then returned toward control levels, and clearance of PAH was unchanged. These data demonstrate an important role for aldosterone and the renin-angiotensin system in the retention of sodium and in ascites formation in dogs with thoracic caval constriction.

THE renin-angiotensin-aldosterone system plays an important role in the pathogenesis of congestive heart failure (Davis, 1965). This was first suggested many years ago by such findings as increased activity of the renin-angiotensin system, hyperaldosteronemia, and increased urinary excretion of aldosterone in both animal models and patients with heart failure; also, bilateral adrenalectomy produced a striking natriuresis in dogs with thoracic caval constriction, a model of low output heart failure (Davis et al., 1953). Furthermore, in these adrenalectomized dogs, reinstition of replacement hormone therapy produced sodium retention that was related quantitatively to the amount of mineralocorticoid given. Johnson and Davis (1973a, 1973b) and, later, Watkins et al. (1976) demonstrated that acute angiotensin blockade reduced arterial pressure and aldosterone secretion in dogs with thoracic caval constriction. In addition, Watkins et al. (1976) were able to attenuate markedly the response to caval constriction by infusion of SQ 20881 for 3 days in conscious dogs; arterial pressure fell to a level of 65 mm Hg, plasma aldosterone...
concentration increased only slightly and not until day 3 and no edema or ascites was detected. There are, however, no reported observations on either the acute response or the chronic daily response to the new orally active converting enzyme inhibitor, SQ 14225, in patients or in animal models of heart failure. SQ 14225 (2-β-methyl-3-mercaptopropionyl-L-proline) (Ondetti et al., 1977) has great potential importance in the treatment of patients with overactivity of the renin-angiotensin system (Gavras et al., 1978). The present observations were undertaken to study the acute response to the initial oral dose of SQ 14225 and the daily responses for 3–4 days to this agent in conscious dogs with thoracic caval constriction and ascites.

**Methods**

Nine female mongrel dogs, weighing 15–21 kg, were used in this study. Chronic indwelling catheters were placed in the femoral artery and femoral vein, routed subcutaneously to the dorsal neck area, and exteriorized. Several days later, a constricting ligature was placed around the thoracic inferior vena cava to produce ascites. The dogs were housed individually in metabolic cages for collection of urine and measurement of electrolyte excretion daily. All dogs ingested a diet that provided 35 mEq of sodium and 25 mEq of potassium daily; water was available ad libitum. The dogs were fed in the late afternoon, and acute experiments were performed the following morning with the dog in the postabsorptive state. All experiments were performed on conscious dogs trained to lie quietly on a padded table.

Positive sodium balances were observed for at least 4–5 days after constriction of the inferior vena cava; there was marked sodium retention and ascites formation. Two days of control measurements of mean arterial pressure, plasma aldosterone concentration (PAC), plasma renin activity (PRA), and plasma electrolytes were also made during this time. The pressor responses to exogenous angiotensin I (2 and 4 μg) were determined during this period.

After these control observations, the dogs were brought to the laboratory so we might study the acute response to the initial dose of SQ 14225. Two 45-minute periods of renal clearance of creatinine (Cr) and para-aminomphippurate (CPAH) were performed in 15% and 40% ethylene glycol-water as the stationary phase. The other adrenal corticoids were eluted first with 15 and 40% ethylacetate/iso-octane, and the aldosterone then was eluted with 60% ethylacetate/iso-octane. Aldosterone was quantified by radioimmunoassay (Bühler et al., 1974). Plasma aldosterone concentration was measured by the method of Bühler, Sealey, and Laragh (1974). Briefly, 2-ml samples of plasma were extracted with methylene chloride. The plasma extract was placed on small celite columns with 40% ethylene glycol-water as the stationary phase. The other adrenal corticoids were eluted first with 15 and 40% ethylacetate/iso-octane, and the aldosterone then was eluted with 60% ethylacetate/iso-octane. Aldosterone was quantified by radioimmunoassay (Bühler et al., 1974). Plasma was prepared for determination of PRA in the following manner: plasma samples were dialyzed against phosphate buffer (pH 5.3) for 18 hours (3 changes). After sodium chloride and diisopropylfluorophosphate were added, the samples were incubated at 37°C for 60 minutes to generate angiotensin I. The reaction then was stopped by placing the samples in a bath of ice water. Angiotensin I content was quantified by radioimmunoassay (Sealey et al., 1974) and PRA ex-
pressed as nanograms of angiotensin I per milliliter per hour. Plasma and urinary levels of creatinine and PAH were determined by standard methods; plasma and urinary electrolytes were measured by flame photometry.

Data were analyzed via analysis of variance and the least significant difference (LSD) statistic; differences at the 5% level are considered statistically significant. When appropriate, Student's t-test was used. Values are presented as means ± SEM.

Results

All dogs used in this study retained sodium and developed ascites. The acute changes in PAC and sodium excretion in response to the initial 10 mg/kg dose of SQ 14225 are shown in Figure 1. Average control values for the two control periods of PAC were 22.7 ± 4.3 and 29.9 ± 8.2 ng%; sodium excretion for the two control periods was 1.9 ± 0.4 and 1.9 ± 0.5 μEq/min. After the administration of SQ 14225, the average PAC value for the group was unchanged for 22.5 minutes, but it then decreased significantly to 10.7 ± 5.0, 11.9 ± 3.6, and 11.0 ± 3.0 ng% after 67.5, 112.5, and 157.5 minutes (Fig. 1, bottom panel). After drug administration, sodium excretion averaged 6.6 ± 3.4, 19.9 ± 10.2, 22.4 ± 11.2, and 17.8 ± 7.9 μEq/min for the four clearance periods. Three dogs showed a natriuretic response after administration of SQ 14225 (Fig. 1, top panel), but three other dogs showed little or no change in sodium excretion. For the group of six dogs, the increase in sodium excretion occurred, despite simultaneous average decreases in arterial pressure and Cc produced by the drug (Table 1). Mean arterial pressure fell from 107-108 mm Hg to 76-80 mm Hg (P < 0.05) and remained at this level throughout the acute experiment; Cc fell from 45-47 ml/min to 35-39 ml/min. However, in two of the three dogs that failed to have a natriuresis, Cc fell to very low levels of 17-29 ml/min, and arterial pressure decreased to 50 and to 70 mm Hg in response to SQ 14225; in the third dog in which sodium excretion failed to increase, arterial pressure fell from 110 to 80 mm Hg, and Cc decreased from 53 to 41 ml/min. For the six dogs (Table 1), the clearance of PAH was unchanged following SQ 14225 administration. Filtration fraction decreased, and PRA increased (P < 0.05) after SQ 14225 administration; PRA remained elevated throughout the acute study. The pressor response to 2 μg of angiotensin I was abolished completely 3 hours after SQ 14225 in five of the six dogs; in the sixth dog, a response amounting to 8 mm Hg was observed.

Data are presented in Table 2 for six dogs before, during, and after chronic administration of SQ 14225 (35 mg/kg per day) for 3 or 4 days. Daily urinary sodium excretion was increased significantly on days 3 and 4 of drug administration and reached maximal natriuresis during these 2 days (Table 2). This natriuresis was associated with what

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Acute Response to SQ 14225 in Conscious Dogs with Thoracic Cava Constriction (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minutes</td>
<td>MAP</td>
</tr>
<tr>
<td></td>
<td>(mm Hg)</td>
</tr>
<tr>
<td>45</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>90</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>135</td>
<td>80 ± 6*</td>
</tr>
<tr>
<td>180</td>
<td>76 ± 6*</td>
</tr>
<tr>
<td>225</td>
<td>77 ± 6*</td>
</tr>
<tr>
<td>270</td>
<td>76 ± 6*</td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure; FF = filtration fraction.

* P < 0.05.
appeared to be a progressive, but statistically insignificant fall in the 8 a.m. levels of PAC during SQ 14225 administration. However, on the 4th day of SQ 14225 treatment, PAC averaged 10 ± 4 ng%, and this value for PAC is not significantly different (P > 0.05) from values of 6.0 ± 1.2 ng% for normal dogs. Plasma electrolyte concentrations were unchanged throughout administration of SQ 14225.

Two of the dogs developed a negative sodium balance during chronic treatment with SQ 14225 (Table 3). Prior to initiation of SQ 14225 administration, daily sodium excretion was less than 3 mEq in both dogs. During SQ 14225 administration, sodium excretion increased promptly and exceeded the sodium intake of 35 mEq/day on days 3 and 4 of the drug. On days 3 and 4 of SQ 14225 administration, sodium excretion increased to 42 and 52 mEq in dog 1 and increased to 91 and 60 mEq in dog 2. PAC fell to the very low levels of 1.5 and 1.6 ng% in dog 1 and 2.5 and 7.5 ng% in dog 2 on days 3 and 4 of SQ 14225 when sodium balance became negative. Arterial pressure decreased only slightly in the two dogs to a level of 95-105 mm Hg during the 4 days of SQ 14225.

Because 8 a.m. levels of plasma aldosterone were not decreased consistently a second measurement of PAC was made 1-2 hours after the 8:30 a.m. dose of SQ 14225 (Fig. 2). Six observations were made in four dogs. Two of the dogs had very low aldosterone concentrations of 1.6 and 2.2 ng% prior to SQ 14225, and PAC did not change following drug administration. For the other four observations, PAC was high (range: 10-54 ng%) prior to SQ 14225 and decreased in each dog after the SQ 14225 (range: 4.7-11 ng%). Prior to initiation of SQ 14225 administration, the intravenous injection of 2 and 4 μg angiotensin I gave an average pressor response of 15.3 ± 1.4 mm Hg and 24.2 ± 2.0 mm Hg, respectively. After initi-

### Table 2: Chronic Response to SQ 14225 in Conscious Dogs with Thoracic Caval Constriction

<table>
<thead>
<tr>
<th>Days</th>
<th>MAP (mMg)</th>
<th>Cn⁺ (ml/min)</th>
<th>CMAH⁺ (ml/min)</th>
<th>FF⁺ (%)</th>
<th>UVₑₑ (mEq/day)</th>
<th>PAC (ng%)</th>
<th>PRA (ng/ml per hr)</th>
<th>Pn⁺ (mEq/liter)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110 ± 3</td>
<td>61 ± 6</td>
<td>167 ± 24</td>
<td>37 ± 4</td>
<td>2.9 ± 1.2</td>
<td>50 ± 15</td>
<td>9.4 ± 2.5</td>
<td>141 ± 1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>109 ± 3</td>
<td>61 ± 6</td>
<td>167 ± 24</td>
<td>37 ± 4</td>
<td>2.0 ± 0.6</td>
<td>32 ± 10</td>
<td>8.2 ± 2.0</td>
<td>141 ± 2</td>
<td>6</td>
</tr>
</tbody>
</table>

**Start SQ 14225 (35 mg/kg per day, orally)**

3 85 ± 5

4 91 ± 2

5 94 ± 5

6 96 ± 4

7 103 ± 2

8 106 ± 2

9 110 ± 3

**Stop SQ 14225 administration**

<table>
<thead>
<tr>
<th>Days</th>
<th>UVₑₑ (mEq/day)</th>
<th>PAC (ng%)</th>
<th>MAP (mMg)</th>
<th>UVₑₑ (mEq/day)</th>
<th>PAC (ng%)</th>
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<tbody>
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<td>3.3</td>
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<td>3.3</td>
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<tr>
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<td>2.9</td>
<td>110</td>
<td>2.9</td>
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<tr>
<td>6</td>
<td>110</td>
<td>2.9</td>
<td>110</td>
<td>2.9</td>
<td>150</td>
</tr>
</tbody>
</table>

### Table 3: The Two Dogs with the Best Natriuretic Responses to SQ 14225

<table>
<thead>
<tr>
<th>Days</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 1</th>
<th>Dog 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>115</td>
<td>3.3</td>
<td>115</td>
<td>3.3</td>
<td>50</td>
<td>50</td>
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<td>2</td>
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<td>110</td>
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<td>2.9</td>
<td>110</td>
<td>2.9</td>
<td>150</td>
<td>150</td>
</tr>
</tbody>
</table>

**Start SQ 14225 (35 mg/kg per day orally)**

8 100 95 11 6.6 10 13

9 95 100 16 19 6.7 2.2

10 105 95 42 91 1.5 2.5

11 105 105 52 60 1.5 7.5

**Stop SQ 14225 administration**

12 105 105 65 2.7 9.2 19

13 110 105 25 0.8 5.8 17

14 110 105 5.4 0.9 8.5 8.0

15 — — 5.0 — 5.8
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PAC (ng\%)

BEFORE SQ14,225 (8:00 A.M.)

1-2 HOURS AFTER SQ14,225

FIGURE 2  PAC in conscious dogs one-half hour before
and 1-2 hours after the oral morning daily dose (10 mg/kg) of the converting enzyme inhibitor, SQ 14225.

Discussion

The important role of aldosterone in the pathogenesis of heart failure was recognized many years ago. In 1950, Deming and Luetscher reported that extracts of urine from patients with congestive failure showed increased sodium-retaining activity. Within the next few years, it was realized that this increased activity reflected an increase in plasma aldosterone and hyperaldosteronuria. The first definitive evidence that increased mineralocorticoid activity of the adrenal cortex produced sodium retention in experimental heart failure came from studies of the effects of bilateral adrenalectomy in dogs with thoracic caval constriction (Davis et al., 1953) and in dogs with severe pulmonic stenosis (Davis et al., 1955). Adrenalectomy led to a striking natriuresis in the absence of hormone therapy, and administration of deoxycorticosterone acetate (DOCA) demonstrated that the degree of sodium retention was related closely to the daily dose of DOCA given. In 1959, it was demonstrated (Yankopoulos et al., 1959) that the major controlling factor for aldosterone secretion is humoral; cross-circulation of blood from dogs with caval constriction and ascites through normal isolated adrenals produced a striking increase in aldosterone secretion in every experiment. In 1961, it was demonstrated (Davis et al., 1961) that this aldosterone-stimulating hormone is secreted by the kidney, and fractionation of crude kidney extracts proved that the active agent is renin (Davis et al., 1962). At the same time, synthetic angiotensin II was found to have potent aldosterone-stimulating activity (Davis, 1961; Genest et al., 1960; Laragh et al., 1960).

In the present study, acute blockade of the renin-angiotensin system with SQ 14225 elicited an increase in sodium excretion in association with a decrease in plasma aldosterone concentration in conscious dogs with thoracic caval constriction. Furthermore, this acute increase in sodium excretion occurred despite a fall in the blood pressure and the glomerular filtration rate which attenuated the natriuresis. However, CoP, and arterial pressure decreased precipitously in two dogs and fell substantially in a third; these changes might have prevented the increase in sodium excretion following SQ 14225. These data suggest that aldosterone is an important regulator of sodium reabsorption in the dog with thoracic caval constriction and indicate that a natriuresis failed to occur in the presence of low levels of arterial pressure and glomerular filtration rate. Both factors are well known to be antinatriuretic.

The decrease in the filtration fraction that occurred in response to the initial dose of SQ 14225 also might have contributed to the natriuresis. Intrarenal arterial infusions of the angiotensin II antagonist, saralasin, into anesthetized dogs with thoracic caval constriction decreased the filtration fraction (Freeman et al., 1975), but sodium excretion failed to increase in these experiments. Plasma aldosterone levels were not measured in these earlier studies (Freeman et al., 1975) in which small doses of saralasin (0.2 and 2.0 µg/kg per minute) were infused locally into the kidney for only short time periods of 40 minutes each. Under these experimental conditions of local saralasin infusion, however, it seems unlikely that aldosterone levels would decrease sufficiently to elicit a natriuresis. The decrease in filtration fraction indicates a predominant postglomerular locus for angiotensin action. Hall et al. (1977) have suggested that angiotensin acts at the efferent arteriolar level to control glomerular filtration rate and, secondarily, renal sodium excretion.

Chronic blockade of the renin-angiotensin system also produced an increase in renal sodium excretion in association with a decrease in plasma aldosterone in the present study. Natriuresis occurred, although complete suppression of aldosterone release was not achieved in each dog at all times with SQ 14225; PAC decreased further within 1-2 hours after the morning dose of SQ 14225 in four of six observations. This low level of PAC could have been accompanied by a transient increase in sodium excretion, which could account for a daily increase in sodium output in the presence of a high PAC value ob-
served before the 8 a.m. dose of SQ 14225. Also, natriuresis occurred in these chronic studies with SQ 14225, although the blood pressure and glomerular filtration rate showed a decrease. Two of the dogs developed a negative sodium balance; in both, PAC fell to very low levels (less than 2.5 ng%). Also, SQ 14225 decreased mean arterial pressure only by about 110 mm Hg. Thus, a negative sodium balance developed in both dogs in association with very low plasma aldosterone levels and a near normal blood pressure.

It is not clear why these two dogs responded better than the remaining ones. It should be mentioned, however, that the degree of constriction of the inferior vena cava varied greatly among the dogs; consequently, dogs with less constriction and presumably with less stimulus for ascites formation might conceivably respond better than those with severe caval constriction. The wide differences in PAC values at 8 a.m. may also be explicable, at least in part, by the differences in the control state among the dogs.

The present findings are consistent with observations in earlier studies (Davis et al., 1955) in which bilateral adrenalectomy produced a striking fluid and electrolyte retention in dogs with thoracic caval constriction. Also, Watkins et al. (1976) reported that infusion of converting enzyme inhibitor, SQ 20881, for 3 days into conscious dogs with thoracic caval constriction prevented the formation of edema and ascites and an increase in PAC. It also has been suggested (McCaa et al., 1978) that chronic administration of SQ 14225 may decrease arterial pressure and increase sodium excretion, in part, by altering plasma levels of kinins and angiotensins. Therefore, the results of the present study are consistent with earlier reports (see review by Davis, 1965) that the renin-angiotensin-aldosterone system provides an important mechanism for the retention of sodium in dogs with thoracic caval constriction. Also, the present data suggest strongly that chronic sodium retention in this experimental model of low output failure is mediated by the aldosterone-stimulated tubular reabsorption of sodium.

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References


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