Effects of an Angiotensin I-Converting Enzyme Inhibitor (SA-446) on Renal Function in Dogs

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Abstract—The effects of an orally active inhibitor of angiotensin-converting enzyme (SA-446) on systemic arterial pressure, renal function and renin release were examined in anesthetized dogs. Intrarenal infusion of the larger dose of SA-446 (0.1 mg/min) caused an increase in RBF, urine flow and renin release and caused a fall in blood pressure. The smaller dose of SA-446 (0.02 mg/min) did not affect the blood pressure, but it increased the urine flow. However, the same dose of SA-446 in combination with probenecid caused a significant fall in blood pressure. The potentiation of SA-446 with probenecid may be explained by the elevation in plasma SA-446 concentration via the inhibition of its tubular secretion by probenecid.

SA-446 ((2R, 4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidine-carboxylic acid) is a newly developed converting enzyme inhibitor (1) which has an antihypertensive effect in laboratory animals (2). The antihypertensive mechanisms of SA-446 seem to be related to the inhibition of the conversion of angiotensin I to the physiologically active pressor octapeptide, angiotensin II, and the potentiation of biological action of bradykinin. However, there are few reports on the effects of the angiotensin-converting enzyme inhibitors on renal function, urine flow, and urinary excretion of sodium—all major determinants of blood pressure. The present study was designed to determine the effects of SA-446 on renal hemodynamics, urine formation, and renin release in anesthetized dogs. Since we have previously reported that SA-446 was excreted via organic anion transport systems (3), the modification of the renal action of SA-446 in the presence or absence of probenecid, a competitive inhibitor of organic anion secretory transport (4), was also examined.

Experiments were carried out using mongrel dogs of both sexes weighing 10–14 kg which had been maintained on standard laboratory chow for 1 week. All dogs were

deprived of food for 24 hr before the experiments were begun. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The left kidney was exposed through a retroperitoneal flank incision. The kidney was carefully denervated, and renal blood flow (RBF) was measured by an electromagnetic flow meter. A 23-gauge needle was inserted into the left renal artery proximal to the flow probe for intrarenal infusion of saline and SA-446 at a rate of 0.5 ml/min. Glomerular filtration rate (GFR) was measured by the creatinine clearance. A polyethylene catheter was inserted into the left ureter for urine collection.

After completion of surgery, the dog was left alone for 60–90 min to allow for stabilization of systemic blood pressure, RBF, and urine flow. Urine was then collected during three consecutive 10-min control clearance periods. At the midpoint of each period, systemic arterial and renal venous blood was collected from the right brachial artery and from the left renal vein via a catheter inserted through the left spermatic or ovarian vein. After the control periods, SA-446 was infused into the renal artery for 30 min in doses of 0.1 and 0.02 mg/min. Urine and blood samples were collected for three

consecutive 10-min clearance periods. Following the 30 min infusion of SA-446, saline was infused into the renal artery to assess the effectiveness of the drug. In 5 dogs, SA-446 was administered at a rate of 0.02 mg/min for 30 min, and urine and blood samples were taken in the same way as described above. In another 5 dogs, a priming dose of probenecid (30 mg/kg) was injected intravenously, followed by a sustaining dose at a rate of 20 mg/kg·hr. Blood samples were obtained at 30 min after starting the administration of probenecid, and urine samples were obtained between 25 and 35 min after the initiation of infusion of probenecid. Then SA-446 was infused into the renal artery for 30 min at a rate of 0.02 mg/min, and urine and blood samples were collected for three consecutive 10-min clearance periods. Plasma renin activity (PRA) was determined by radioimmunoassay of angiotensin I (5) and expressed as nano-

grams of angiotensin I per milliliter per hour.

Table 1 shows the effects of intrarenal infusion of SA-446 on renal hemodynamics, urine flow and renal venous PRA. SA-446 infusion at a rate of 0.1 mg/min resulted in a significant increase in RBF, urine flow and urinary excretion of sodium and in a significant fall in systemic arterial pressure. These responses reached a maximum about 20 min after the start of infusion and remained at the same level for the consecutive periods. Urine flow and urinary excretion of sodium increased approximately 50% without any significant change in GFR. Data are not shown in Table 1, but osmolar clearance and free water reabsorption increased almost proportionally. Renal venous PRA gradually increased and the calculated renin secretion rate increased about 2- to 3-fold.

Table 2 shows the renal effects of the smaller dose of SA-446 and the effects of SA-446 infusion in combination with pro-

Table 1. Effects of the larger dose of SA-446 on renal function and renal venous PRA (n=8)

	Time min	BP mmHg	RBF ml/g·min	GFR ml/g·min	UF μl/g·min	U _{Na} V μEq/g·min	PRA ng/ml·hr
Control	0	124±3	3.17±0.34	0.74±0.06	20±4	3.4±0.7	2.1±0.6
SA-446	10	115±6*	3.56±0.41*	0.73±0.06	30±5	5.2±1.1*	4.5±1.6*
(0.1 mg/min)	20	113±5*	3.62±0.40*	0.71±0.05	32±5*	5.4±1.0*	4.4±1.5*
	30	111±5*	3.65±0.39*	0.73±0.06	31±4*	5.1±0.9	5.0±1.9*

All values are means±S.E. n=number of dogs tested, BP=systemic arterial pressure, RBF=renal blood flow, GFR=glomerular filtration rate, UF=urine flow, U_{Na}V=urinary excretion of sodium, PRA=plasma renin activity in renal venous blood. *indicates significance of the difference between control and experimental periods (P<0.05).

Table 2. Effects of the smaller dose of SA-446 on renal function and renal venous PRA before and after treatment of probenecid (n=5)

	Time min	BP mmHg	RBF ml/g·min	GFR ml/g·min	UF μl/g·min	U _{Na} V μEq/g·min	PRA ng/ml·hr
Control	0	128±9	2.98±0.27	0.73±0.05	8±1	1.7±0.3	2.1±0.4
SA-446	10	128±9	3.20±0.29	0.73±0.07	13±3	3.0±0.8	2.0±0.4
(0.02 mg/min)	20	124±9	3.52±0.31*	0.75±0.08	18±5*	3.9±1.1*	3.0±1.0
	30	123±9	3.67±0.36*	0.73±0.08	20±6*	4.2±1.3*	3.3±1.1
Control	-35	125±4	3.57±0.25	0.72±0.08	14±5	3.6±1.1	3.3±1.0
Probenecid	0	128±5	3.73±0.28	0.74±0.09	18±6	4.1±1.4	3.3±1.0
SA-446	10	121±5	4.61±0.25*	0.78±0.06	25±8*	5.6±1.6	5.5±2.2
(0.02 mg/min)	20	117±6*	4.80±0.20*	0.79±0.08	27±9*	5.9±1.8*	6.4±2.5*
	30	115±6*	4.89±0.29*	0.85±0.07	24±9*	5.2±1.5	9.2±4.1*

All values are means±S.E. n=number of dogs tested. *indicates significance of the difference (P<0.05). See legend to Table 1 for abbreviations.

benecid. The smaller dose of SA-446_i (0.02 mg/min) slightly decreased the systemic arterial pressure and slightly increased the renal venous PRA. These changes were not statistically significant. However, RBF, urine flow and urinary excretion of sodium significantly increased. Intravenous administration of probenecid in a dose of 30 mg/kg and at a rate of 20 mg/kg·hr in itself did not affect RBF, GFR, urine flow and renal venous PRA. Infusing the same dose of SA-446 during probenecid infusion resulted in a significant fall in systemic arterial pressure and in a significant increase in renal venous PRA in contrast to the results observed in the absence of probenecid. Urine flow and urinary excretion of sodium were also increased by the SA-446 infusion in combination with probenecid, but these changes were not significantly different from those recorded when only SA-446 was administered.

SA-446, a newly-synthesized sulfhydryl compound, inhibited the angiotensin-converting enzyme purified from rabbit lung. The inhibitory action was 4–5 times greater than that of captopril (2). The larger dose of SA-446 (0.1 mg/min) caused an increase in RBF, urine flow, and renin release. Systemic arterial pressure decreased about 15 mmHg 30 min after the start of infusion. Urine flow and urinary excretion of sodium increased without any change in GFR. Osmolar clearance and free water reabsorption increased proportionally, indicating that SA-446 has little effect on the medullary portion of the ascending limb of Henle and may have a proximal site of action. Bailie and Barbour (6) and we (7) reported similar results with SQ 20,881 and YS-980 which have inhibitory action on an angiotensin-converting enzyme. The smaller dose of SA-446 (0.02 mg/min) also caused an increase in RBF, urine flow and urinary excretion of sodium, but did not affect the systemic arterial pressure and renal venous PRA. The same dose of SA-446 in combination with probenecid caused a significant fall in blood pressure. However, the changes in urine flow and in the urinary excretion of sodium were not modified by probenecid. Since converting enzyme inhibitors such as captopril and SA-

446 are weak organic anions (3, 8), and SA-446 has a structural resemblance to penicillin, the renal excretion of SA-446 is markedly inhibited by probenecid (3). The potentiation of its hypotensive action may be explained by the elevation in plasma SA-446 concentration via the inhibition of its tubular secretion by probenecid. However, it is difficult to explain why the effects of SA-446 on the urine flow and the urinary excretion of sodium were not potentiated by probenecid. In general, the hypotension should induce antidiuresis. Thus, it can be considered that the potentiatory actions of probenecid on urine formation might be masked by the hypotension observed in the SA-446 infusion in combination with probenecid.

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