Studies on Mechanisms of Antinephritic Action of SA-446, an Angiotensin I Converting Enzyme Inhibitor (1) A Comparison with Actions of Spironolactone, Kallidinogenase and Saralacin

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Abstract—The present study was made to clarify the mechanisms of the antinephritic action of SA-446, an angiotensin I converting enzyme inhibitor, on crescentic-type anti-GBM nephritis in rats as compared to the actions of spironolactone (an antialdosterone agent), kallidinogenase (a kallikrein agent) and saralasin (an angiotensin II antagonist). SA-446 (25 mg/kg/day, p.o.) had a tendency to reduce the urinary protein excretion and plasma urea nitrogen content. In addition, this drug remarkably inhibited not only glomerular histopathological changes (i.e., crescent formation, the adhesion of capillary walls to Bowman's capsule and fibrinoid necrosis) but also the elevation of blood pressure. Spironolactone (25 mg/kg/day, p.o.) and kallidinogenase (25 KU/day, i.m.) also showed beneficial effects on glomerular histopathological changes and hypertension, although both drugs were not as effective as SA-446. However, saralasin (72 μ g/day, s.c.) caused a marked aggravating action on this nephritis. This nephritic model showed a marked low activity of plasma renin all through the 40 day experimental period. In this model, the urinary aldosterone excretion was increased, in spite of the decrease in plasma renin activity. SA-446 and kallidinogenase significantly inhibited the decrease in plasma renin activity and the increase in urinary aldosterone excretion. Spironolactone inhibited only the increase in the aldosterone excretion. However, saralasin decreased the plasma renin activity under the control level and strongly increased the urinary aldosterone excretion (about 1.8 times the control level on the 20th day). These results suggest that the antinephritic effect of SA-446 may be related to the antihypertensive action and the increase in renal blood flow through activation of the kallikrein-kinin and prostaglandins systems.

In recent years, it has been demonstrated that an angiotensin I converting enzyme (ACE) inhibitor, a new type of hypotensive agent, reduces the blood pressure in renovascular hypertension as well as essential hypertension (1). It has been speculated that the hypotensive action of ACE inhibitors may be caused by inhibition of the conversion of angiotensin I to angiotensin II and/or by inhibition of the destruction of kinins, the potent vasodepressors (2-4). We have already reported that a positive correlation

exists between the crescent formation in glomeruli and the blood pressure in crescentic-type anti-glomerular basement membrane (anti-GBM) nephritis of rats and that SA-446 [(2R, 4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidine-carboxylic acid], an ACE inhibitor, shows beneficial effects on histopathological changes in glomeruli and hypertension (5). This result suggests that either the renin-angiotensin-aldosterone or kallikrein-kinin (K-K) system may be related to the pro-

gression of glomerular histopathological changes and the elevation of blood pressure in this nephritic model. In the present study, the mechanisms of antinephritic action of SA-446 were investigated by comparing the actions of spironolactone, an antialdosterone agent; kallidinogenase, a kallikrein agent; and saralasin, an angiotensin II antagonist.

Materials and Methods

Animals: Male Sprague-Dawley strain rats weighing approx. 190 g (Keari Chubu Branch Office) were used. These animals were housed in an air-conditioned room at $23 \pm 1^\circ\text{C}$.

Drugs: Drugs used were SA-446 (Santen Pharmaceutical Co.), spironolactone (Dainippon Pharmaceutical Co.), saralasin (Peptide Research Laboratory) and kallidinogenase [Depo-Kallikrein] (Bayer Co.). Both SA-446 and spironolactone were suspended in 0.5% gum arabic. Saralasin and kallidinogenase were dissolved in 0.9% saline and 10% polyvinylpyrrolidone, respectively.

Induction of nephritis and drug administration: In experiment I, crescentic-type anti-GBM nephritis was induced in rats by a slight modification of the method reported previously (5). The rats were injected with 0.5 ml of rabbit anti-rat GBM serum (anti-GBM serum)/animal into the tail vein. On the day after the anti-GBM serum injection, 24 hr urine samples were collected, and the rats were then divided into three groups ($n=8$), so that the average protein content in the 24 hr urine sample of each group was at the same level. After grouping, these animals were injected with 5 mg of rabbit γ -globulin in 0.25 ml of Freund's complete adjuvant into the hind foot-pads. Two groups were given p.o. daily 25 mg/kg of SA-446 and spironolactone from the day of rabbit γ -globulin injection (the 2nd day) to the 39th day. The remaining group was given vehicle (0.5% gum arabic, p.o., daily) as the control. In experiment II, nephritic rats were divided into three groups ($n=7$). Two groups were used for test drugs. One group was given 25 KU of kallidinogenase/animal, i.m., daily, and the other group was injected s.c. continually with 72 $\mu\text{g/day}$ (50 $\mu\text{g/min}$) of saralasin with a mini-osmotic pump for the same period as in experiment I. The remaining

group was given the vehicle (0.9% NaCl, i.m., daily) as the control.

Collections of urine, blood and kidneys: The 24 hr urine samples were collected on the 1st, 5th, 10th, 20th, 30th and 40th days after the injection of anti-GBM serum. Blood was drawn in a volume of 0.5 ml from the tail vein on day 0 and the 7th, 21st and 38th days. On the 40th day, whole blood was taken from the abdominal aorta. In the case of the urine collection, each animal received 5 ml of distilled water twice orally without feeding at the beginning and 5 hr after the collection. The 24 hr urine samples were obtained by keeping each animal in individual metabolic cages. The urine obtained was then centrifuged at 3,000 rpm for 10 min at 4°C , and the supernatant was used for the determinations. In the case of blood collection, blood was taken in centrifuge tubes containing 1 mg of EDTA-2 Na (1–5% solution in 0.9% NaCl)/ml of blood. The blood was then centrifuged at 3,000 rpm for 10 min at 4°C to obtain plasma. After whole blood collections on the 40th day, both kidneys were taken for histopathological studies.

Determinations of urinary protein and plasma urea nitrogen contents: The urinary protein content was determined by the method of Kingsbury et al. (6) and expressed as mg/24 hr urine. The plasma urea nitrogen content was determined in accordance with the method of Searcy and Cox (7) and expressed as mg/dl of plasma.

Determinations of plasma renin activity and urinary aldosterone contents: The plasma renin activity was determined with a commercial kit [RENCTK] (Midori Juji) by radioimmunoassay (8) and expressed as ng angiotensin I (Ag I)/ml of plasma/hr. The urinary aldosterone content was determined with an aldosterone-1-125 kit [ALDOCTK-125] (Midori Juji) by radioimmunoassay (9) and expressed as ng/24 hr urine.

Measurement of blood pressure: Blood pressure was measured by a tail plethysmography [KN-209] (Natsume) on day 0 and the 7th, 14th, 21st, 28th and 38th days.

Assessment of histopathological parameters: For light microscopic study, kidneys were dehydrated and fixed by immersing the tissues stepwise into low to high concen-

tations of alcohol. The tissues were then embedded in paraffin and sectioned 2–3 μ m thick. The sections were stained with hematoxylin and eosin and Masson trichrome. As histopathological parameters in the glomeruli, crescent formation, adhesion of capillary walls to Bowman's capsule (adhesion) and fibrinoid necrosis were observed in fifty glomeruli per section under light microscopy. The degree of changes in these three histopathological parameters was scored as: mild (1), moderate (2) and severe (3). The number of glomeruli corresponding to each score was represented as n_1 , n_2 and n_3 . The crescent formation index (CI), the adhesion index (AI) and the fibrinoid necrosis index (FI) were calculated by the following formula: CI, AI

and $FI = 1 \times n_1 + 2 \times n_2 + 3 \times n_3$. The degree of glomerular lesions was expressed as the index of glomerular lesions (IGL) and calculated by the following formula:

$$= \frac{(3 \times CI) + (2 \times AI) + (1 \times FI)}{(3 + 2 + 1) \times 50}$$

Statistical analysis: The data are given as the mean $\pm$ S.E., and the results were statistically evaluated by Student's *t*-test.

Results

1. Effects on urinary protein excretion and plasma urea nitrogen level (Fig. 1)

SA-446 and spironolactone: SA-446 (25 mg/kg/day, p.o.) significantly inhibited the urinary protein excretion by 30.8% on the 10th day and then tended to inhibit it up to

Fig. 1. Effects of SA-446, spironolactone, kallidinogenase and saralasin on urinary protein excretion and plasma urea nitrogen content in crescentic-type anti-GBM nephritis in rats. Each plot denotes the mean value with S.E. of 7 or 8 rats. The number in parenthesis indicates the percent inhibition which was derived from the following formula: $\frac{C-T}{C-N} \times 100$ (C: Control, T: Test drug, N: Normal). *indicates a significant difference from the control at $P < 0.05$.

Photo 1. Kidney sections from control (a), SA-446, 25 mg/kg/day, p.o.-treated (b) and spironolactone, 25 mg/kg/day, p.o.-treated (c) rats on the 40th day after injection of anti-GBM serum. Masson trichrome stain ($\times 200$).

Photo 2. Kidney sections from kallidinogenase, 25 KU/day, p.o.-treated (a) and saralasin, 72 μ g/day, s.c.-treated (b) rats on the 40th day after injection of anti-GBM serum. Masson trichrome stain ($\times 200$).

the 40th day. In addition, this drug showed a tendency to reduce the plasma urea nitrogen levels on the 40th day. On the other hand, spironolactone (25 mg/kg/day, p.o.) was ineffective in reducing both parameters.

Kallidinogenase and saralasin: Kallidinogenase (25 KU/day, i.m.) did not affect the urinary protein excretion and the plasma urea nitrogen level. On the other hand, saralasin (72 μ g/day, s.c.) caused a significant increase of 26% on the protein excretion on the 40th day.

2. Effects on histopathological parameters in glomeruli (Fig. 2)

SA-446 and spironolactone: In the histo-

pathological assessment on the 40th day, SA-446 (25 mg/kg/day, p.o.) remarkably reduced the CI, the AI and the FI by 50.3%, 53.4% and 56.5%, respectively. Spironolactone (25 mg/kg/day, p.o.) also significantly reduced the indices of these three histopathological parameters by about 40%. SA-446 and spironolactone reduced the IGL calculated on the basis of these three parameters by 52.0% and 35.7%, respectively. Representative micrographs of glomeruli from rats treated with both drugs are given in Photo 1.

Kallidinogenase and saralasin: Kallidinogenase (25 KU/day, i.m.) was as effective as

Fig. 2. Effects of SA-446, spironolactone, kallidinogenase and saralasin on histopathological parameters in glomeruli on the 40th day after injection of anti-GBM serum in crescentic-type anti-GBM nephritis in rats. Each column denotes the mean $\pm$ S.E. of 7 or 8 rats. The number in parenthesis indicates a percent inhibition which was derived from the following formula: $\frac{C-T}{C} \times 100$ (C: Control, T: Test drug). *, ** and *** indicate a significant difference from the control at $P < 0.05$, 0.01 and 0.001, respectively.

spironolactone on histopathological parameters. However, saralasin (72 µg/day, s.c.) significantly increased the CI, the AI and the FI by 40.6%, 40.5% and 82.0%, respectively. Thus, only saralasin caused an undesirable effect histopathologically. Photo 2 indicates representative micrographs of glomeruli from rats given both drugs.

3. Effects on blood pressure (Fig. 3)

SA-446 and spironolactone: SA-446 (25 mg/kg/day, p.o.) pronouncedly inhibited the elevation of blood pressure by 42.0%–65.6% from the 7th to the 30th days. Spironolactone (25 mg/kg/day, p.o.) also showed an anti-hypertensive effect for the same periods, but

the effect was inferior to that of SA-446.

Kallidinogenase and saralasin: Kallidinogenase (25 KU/day, i.m.) was also as potent as SA-446 in lowering the blood pressure. However, saralasin (72 µg/day, s.c.) markedly elevated the blood pressure over that of the control on the 38th day.

4. Effects on plasma renin activity and urinary aldosterone excretion (Fig. 4)

SA-446 and spironolactone: The plasma renin activity of the control group showed a markedly low level as compared with that of the normal group on the 7th day (0.69 ± 0.1 ng Ag I/ml/hr vs. 8.31 ± 1.45 ng Ag I/ml/hr). Thereafter, the low level of plasma renin

Fig. 3. Effects of SA-446, spironolactone, kallidinogenase and saralasin on blood pressure in crescentic-type anti-GBM nephritis in rats. Each plot denotes the mean value with S.E. of 7 or 8 rats. The number in parenthesis indicates the percent inhibition which was derived from the following formula: $\frac{C-T}{C-N} \times 100$ (C: Control, T: Test drug, N: Normal). * and ** indicate a significant difference from the control at $P < 0.05$ and 0.01 , respectively.

activity of the control group remained unchanged by the 38th day. SA-446 (25 mg/kg/day, p.o.) caused a significant inhibition of 46.8% on the marked lowering of plasma renin activity on the 38th day. However, the renin activity was little affected by spironolactone (25 mg/kg/day, p.o.). The urinary aldosterone excretion of the control group was significantly high as compared with that of the normal group on the 1st day (26.1 ± 1.0 ng/24 hr vs. 19.4 ± 1.4 ng/24 hr). Later, the aldosterone excretion continued to increase up to the 20th day with the level decreasing slightly on the 40th day. The marked increase in urinary aldosterone excretion was significantly inhibited 77.0% and 89.6% by SA-446 and spironolactone on the 20th day, respectively.

Kallidinogenase and saralasin: Like SA-446, kallidinogenase (25 KU/day, i.m.) significantly inhibited the lowering of plasma renin activity by 33.0% on the 38th day and the increase in urinary aldosterone excretion by 57.9% on the 40th day. However, saralasin (72 μ g/day, s.c.) significantly

decreased the renin activity by 12.6% and 23.5% on the 21st and 38th days, respectively, as compared to the control level. In addition, this agent markedly increased the aldosterone excretion by 1.8 and 1.5 times the control levels on the 20th and 40th days, respectively.

Discussion

The present study confirmed that SA-446 (25 mg/kg/day, p.o.), an ACE inhibitor, has a very beneficial effect on histopathological changes in glomeruli and hypertension of crescentic-type anti-GBM nephritis in rats, although it had only a very weak inhibitory effect on urinary protein excretion. In general, there is no correlation between renal histological findings and proteinuria of glomerulonephritis. We previously found that there was a close relationship between glomerular lesions and blood pressure in this model (5). It has been generally considered that the prognosis of patients with hypertensive-type nephritis is incurable as compared with that of patients with normotensive nephritis. Tikkanen et al. (10) demonstrated

Fig. 4. Effects of SA-446, spironolactone, kallidinogenase and saralasin on plasma renin activity and urinary aldosterone excretion in crescentic-type anti-GBM nephritis in rats. Each plot denotes the mean value with S.E. of 7 or 8 rats. The number in parenthesis indicates the percent inhibition which was derived from the following formula: $\frac{C-T}{C-N} \times 100$ (C: Control, T: Test drug, N: Normal). * and ** indicate a significant difference from the control at $P < 0.05$ and 0.01 , respectively.

experimentally that DOCA-NaCl-treated nephritic rats had a heavier proteinuria and more marked renal lesions than NaCl-treated nephritic rats. Nishio et al. (11) also reported that DOCA-NaCl-treated rats injected i.v. with anti-GBM serum caused more marked glomerular lesions and more severe hypertension than only DOCA-NaCl-treated rats. These results suggest that nephritis and hypertension may closely act as an aggravating factor to each other. Recently, we reported that nicardipine, a calcium antagonist which has been widely used as a hypotensive agent, was beneficial for preventing the progression of crescentic-type anti-GBM nephritis in rats (12). Therefore, a potent preventive action of SA-446 on

histopathological changes in glomeruli may be in great part due to the antihypertensive action. Conversely, histopathological improvement by SA-446 may prevent the elevation of blood pressure.

It has been recognized by Iso et al. (13) that the *in vitro* inhibitory action of SA-446 on semi-purified ACE obtained from rabbit lung is 4 to 5 times larger than of captopril. In the *in vivo* assay system, the compound has been also demonstrated to be more potent than captopril in inhibiting the ACE in dogs but not in rats (14). Furthermore, SA-446 given orally has been reported to have marked antihypertensive actions not only on two kidney, one-clip renal hypertensive rats, a high renin model of hypertension (14, 15),

but also on adult spontaneously hypertensive rats, a normotensive or low renin model of hypertension (13). In the present experiment, SA-446 resulted in a potent hypertensive action on crescentic-type anti-GBM nephritis in rats, although this model maintained markedly low activity of plasma renin during the experimental periods. These findings suggest that the depressor action of SA-446 on high renin hypertension may be linked to inhibition of the conversion of angiotensin I to angiotensin II. However, the depressor action of this drug on low renin hypertension such as the nephritic model used in the present study cannot be explained by inhibition of the renin-angiotensin (R-A) system. In the present experiment, the antinephritic effect of saralasin, an angiotensin II receptor blockade, was examined to clarify whether or not SA-446 exerts the antinephritic action by inhibiting the R-A system. If the possibility is considered as one of the mechanisms of the antinephritic action of SA-446, saralasin is also expected to be beneficial on this nephritis. However, saralasin (72 μ g/day, s.c.) brought about severe glomerular lesions and marked pressor action as compared with the control. This observation indicates that the antinephritic action of SA-446 in this nephritic model is not always attributed to inhibition of the R-A system. Furthermore, saralasin, having a chemical structure similar to that of angiotensin II, may act synergistically with angiotensin II on angiotensin II receptors in this model with a low renin activity in plasma because of the decrease in angiotensin II synthesis. This is a reason why saralasin caused the aggravation of the disease.

In the present nephritic model, a marked increase in urinary aldosterone excretion was observed throughout the experimental period, in spite of plasma renin activity being very low. Therefore, the increase in aldosterone secretion observed may not be mediated by activation of the R-A system. Aldosterone is secreted from the zona glomerulosa of the adrenal cortex. However, no histological abnormality of the adrenal cortex was detectable by light microscopic observation (Y. Suzuki et al., unpublished data). At present, the mechanism by which the

aldosterone secretion was increased remains unclear. In the present experiment, we investigated whether or not the increase in the aldosterone secretion may be involved in the progression of this nephritis by administering spironolactone, an antialdosterone agent. Spironolactone (25 mg/kg/day, p.o.) histopathologically revealed a beneficial effect, although the effect was inferior to that of SA-446. In addition, both drugs inhibited the increase in urinary aldosterone excretion. These results suggest that the excess of aldosterone secretion may be in part related to the progression of the disease, and the protective effect of spironolactone on this nephritis may be explained by inhibition of aldosterone receptors.

It has been shown by Glasser and Michael (16) that there is a prompt significant decrease in urinary kallikrein excretion in anti-GBM nephritis of rats. Therefore, the decrease in urinary kallikrein excretion is likewise expected in the present nephritic model. It is reported that a positive correlation exists between urinary kallikrein and aldosterone excretion in normotensive subjects (17). These findings suggest that the cause of hypertension of this nephritic model may be related to the decrease in renal kallikrein release, even though the aldosterone secretion is increased.

As ACE is identical with kininase II which degrades bradykinin, a potent vasodilator, to inactive fragments, the ACE inhibitors can mediate the accumulation of endogenous bradykinin due to kininase II inhibition. In addition, bradykinin stimulates prostaglandins synthesis (18). Kondo et al. (19) reported that following oral administration of SA-446 or captopril, the maximal decrease in mean blood pressure correlated with the increase in plasma bradykinin. If SA-446 acts through activation of the K-K and prostaglandins systems, kallikrein agents may be also effective on this nephritis. In the present experiment, kallikreinogenase (25 KU/day, i.m.), a kallikrein agent, showed beneficial effects on histopathological changes in glomeruli and hypertension in this nephritis. Therefore, it is postulated from the above findings that the antihypertensive action of SA-446 on crescentic-type anti-GBM

nephritis may be mainly attributable to activation of the K-K and prostaglandins systems. SA-446 and kallidinogenase also evoked the elevation of plasma renin activity. This result may be related to activation of the K-K and prostaglandins systems with SA-446 or kallidinogenase.

In recent years, it has been considered that intraglomerular coagulation and subsequent decrease in blood flow of small vessels may play an important role in progression of glomerulonephritis (20, 21). The accumulation of endogenous bradykinin and the increase in prostaglandins by SA-446 may increase the renal blood flow. It is concluded from these results that the antinephritic effect of SA-446 may be due to the reduction in blood pressure and the increase in renal blood flow through activation of the K-K and prostaglandins systems.

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