Beneficial Renal Effects of CS-905, a Novel Dihydropyridine Calcium Blocker, in SHR

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Abstract—CS-905 is a potent dihydropyridine calcium blocker that has a gradual and long-lasting antihypertensive action with little tachycardia in SHR. In this study, we investigated chronic and acute effects of CS-905 on renal functions in SHR. To examine the chronic effects, 23 week-old male SHR were treated with CS-905 (1 or 3 mg/kg/day, p.o.) or 0.3% CMC (carboxymethylcellulose). After the 15 week-treatment, the agent dose-relatedly lowered systolic blood pressure measured 24 hr after the final administration (184±2 and 173±3 mmHg at 1 and 3 mg/kg/day vs. 218±4 mmHg for the control group). Natriuresis and the reduction of urinary protein excretion were also observed in the CS-905 treated groups. Urinary NAG (N-acetyl-Q-D-glucosaminidase) activity tended to decrease, but not significantly. Histopathological changes observed in the SHR kidney were reduced by chronic treatment with CS-905. On a single oral administration in 38 week-old SHR, CS-905 caused natriuresis at a dose of 3 mg/kg, but did not affect urinary protein excretion and urinary NAG activity. These effects of CS-905 on renal functions may be beneficial in the treatment of hypertension.

Hypertension accelerates renal injuries in patients with renal diseases (1, 2), and eighty percent of the patients with chronic glomerulopathies become hypertensive (3). These facts suggest that hypertension and renal failure is closely related. It is therefore important to determine if an antihypertensive agent affects renal functions.

CS-905 is a potent dihydropyridine Ca++ antagonist that has a gradual and long-lasting antihypertensive action with little tachycardia in SHR (4). In this study, we investigated chronic and acute effects of CS-905 on renal functions in SHR, the best model to date of human essential hypertension. In another series of experiments, we measured urinary protein excretion and urinary NAG activity in SHR and age-matched WKY.

Materials and Methods

Chronic effects: Male 23 week-old SHR, weighing 335–355 g, were obtained from Hoshino Laboratory animals (Japan). After systolic blood pressure (SBP) was measured by the tail cuff method (PE-300, Narco Biosystems Inc., TX, U.S.A.), the rats were divided into 3 groups (eight rats for each group) so that SBP were evenly distributed. The animals were treated with CS-905 (1 and 3 mg/kg/day, p.o., suspended in 0.3% CMC) or 0.3% CMC for 15 weeks. Food and water were given ad libitum. At the end of the 15 week dosing, SBP was measured 24 hr after the final administration of the drug or vehicle. On the next morning the rats received both oral saline load at a volume of 2.5 ml/100 g of body weight and drug administration. Immediately after these oral administrations, the individual animals were placed in metabolism cages, and the spontaneous urine was sampled for 0–6 hr and 6–24 hr under deprivation of food and water. The collected urine was submitted to the measurement of urine volume, urinary electrolytes (Na+, K+,
Cl\(^+\)), urinary protein, and urinary NAG activity. Sodium, potassium and chloride were measured by means of a flame photometer (Hitachi 750). Total protein concentration in the urine and urinary NAG activity were determined with a kit purchased from Wako (Micro TP-test) and Shionogi (NAG test), respectively. Blood samples were collected from the tail vein 24 hr after the final administration and assayed for plasma renin activity (PRA) with a kit purchased from Dinabot Laboratories.

**Acute effects:** Male 38 week-old SHR weighing 335–415 g were used. The rats had free access to food and tap water until the morning of the experiment. CS-905 (1 and 3 mg/kg) or 0.3% CMC was administered with physiological saline at a volume of 2.5 ml/100 g. Urine sampling and the measurement of urinary parameters were performed as described above.

**SHR and WKY:** Male 12 week-old SHR (n=10) and age matched WKY (n=10), both provided by Hoshino Laboratory Animals (Japan), were used. All animals had free access to food and water. Urine was collected at 17, 23, 27, 32, 37, 39 and 42 weeks of age as described above. Total urinary protein excretion and urinary NAG activity were measured.

**Histopathological examination:** The kidney was excised, fixed in phosphate-buffered 10% formalin. After embedding in paraffin, specimens were sectioned at a thickness of 3 \(\mu\)m and stained with hematoxylin-eosin, periodic acid-Schiff, Azan and Elastica-van Gieson. The number of injured arteries or glomeruli was counted under the light microscope for the one whole section and divided by the number of all interlobular and arcuate arteries (59–122) or glomeruli (127–193) to give the incidence of injury.

**Results**

Urinary protein excretion and urinary NAG activity were measured in SHR and WKY, and the results were plotted against age in Figs. 1 and 2. Both measurements were significantly higher in SHR than WKY throughout the 25 week observation period. Urinary protein excretion in SHR began to rise at an accelerated rate over 27 weeks (Fig. 1). Urinary NAG activity in SHR were constantly higher than WKY over the whole observation period (Fig. 2).

Table 1 shows systolic blood pressure (SBP) in the control (0.3% CMC), the low and high dose groups (CS-905 at dose of 1 and 3 mg/kg/day, p.o.), before and after the 15 week dosing. Long-term administration of CS-905 in SHR lowered SBP measured 24 hr after the final administration in a dose-related manner.

Table 2 shows urine volume (UV) and the amount of urinary electrolytes excretion (\(\text{UNa}^+\text{V}, \text{UK}^+\text{V}\) and \(\text{UCl}^-\text{V}\)) in the 0–6 hr, 6–24 hr and 0–24 hr periods for the 3 groups after chronic administration of the vehicle or
Table 1. Systolic blood pressure before and after administration of the vehicle or CS-905 for 15 weeks

| Dose (mg/kg) | Before (mmHg) | After (mmHg) |
|-------------|---------------|--------------|
| Control     | 212±5         | 218±5        |
| 1           | 212±4         | 184±2**      |
| CS-905      |               |              |
| 3           | 222±6         | 173±3**      |

All values are the mean±S.E. of 8 rats. **P<0.01, compared with the values before administration.

Table 2. UV, UNa+V, UK+V and UCl−V in the 0–6 hr, 6–24 hr and 0–24 hr period after chronic administration of the vehicle or CS-905

| Dose (mg/kg) | Time (hr) | UV (ml/kg) | UNa+V (μEq/kg) | UK+V (μEq/kg) | UCl−V (μEq/kg) |
|-------------|-----------|------------|----------------|---------------|----------------|
| Control     | 0–6       | 21.9±1.7   | 2610±313       | 870±66        | 2655±305       |
|             | 6–24      | 10.4±1.0   | 1269±231       | 1508±75       | 1350±169       |
|             | Total     | 32.3±2.0   | 3879±379       | 2378±98       | 4004±289       |
| CS-905      | 0–6       | 30.3±1.8** | 3222±182       | 1080±52*      | 3410±164*      |
|             | 6–24      | 9.1±0.6    | 939±85         | 1198±55       | 753±87         |
|             | Total     | 39.4±1.5   | 4161±156       | 2279±60       | 4039±206       |
|             | 0–6       | 35.0±1.1** | 4116±146**     | 1179±41**     | 4350±171**     |
|             | 6–24      | 8.5±0.5    | 788±96         | 1396±67       | 592±56         |
|             | Total     | 43.5±1.1** | 4903±116*      | 2575±92       | 4942±129*      |

All values are the mean±S.E. of 8 rats. *P<0.05, **P<0.01, compared with the values in the control group.

Figures 3 and 4 show the amount of protein excreted and NAG activity for the 0–24 hr urine collection in the 3 groups. CS-905, when administered for 15 weeks, produced a dose-related reduction in urinary protein (Fig. 3). The degrees of decrease in urinary protein excretion were 39 and 48% for the low and high dose groups, respectively, as compared with the control group. Urinary NAG activity in the low and high dose groups tended to decrease, but not significantly (Fig. 4). Plasma renin activity (PRA) was decreased significantly in the high dose group (Fig. 5).

Histological examination of the kidney from the control SHR revealed intimal proliferation of interlobular and arcuate arteries. Glomeruli exhibited severe sclerosis and pericapsular fibrous thickening, while tubules exhibited less severe necrosis, degeneration,
dilatation and protein casts (Fig. 6a, b). Pathological changes in glomeruli were much more marked in the juxtamedullary zone than in the cortex.

Table 3 shows the incidence of arterial and glomerular injuries. Chronic treatment with CS-905 at 3 mg/kg/day significantly de-

Fig. 4. Urinary NAG activity after chronic administration of the vehicle or CS-905.

Fig. 5. Plasma renin activity after chronic administration of the vehicle or CS-905. *P<0.05, compared with the value in the control group.

Fig. 6. Histological examination. (a) Marked intimal proliferation of interlobular arteries and slight glomerular sclerosis in untreated SHR. H.E. ×100. (b) Renal tubular dilatation and protein casts in untreated SHR. H.E. ×50. (c) Arterial and glomerular injuries were reduced in SHR treated with 3 mg/kg CS-905. H.E. ×50. (d) Renal tubular injuries were reduced in SHR treated with 3 mg/kg CS-905. H.E. ×50.
creased these pathological changes (Table 3 and Fig. 6c, d), although improvement at 1 mg/kg/day was not significant (Table 3).

Table 4 summarizes the degree of renal tubular injury. Renal tubules from the control SHR exhibited slight necrosis and degeneration and dilatation. These pathological changes were reduced in the CS-905 groups in a dose-related manner.

On a single oral administration, CS-905 at a dose of 3 mg/kg caused significant increases of UV and UNa+V in the 0–6 hr and 0–24 hr urine collection. UK+V and UCl−V at a dose of 3 mg/kg and all urinary parameters at a dose of 1 mg/kg tended to increase, but not significantly (Table 5). Single oral administrations of CS-905 at both doses did not affect urinary protein excretion and urinary NAG activity: urinary protein at 1 and 3 mg/kg of CS-905 were 74±24 and 83±23 mg/kg/day, respectively, vs. 87±17 mg/kg/day in the control SHR; and urinary NAG activity

### Table 3. Incidence of arterial and glomerular injury in the SHR kidney after chronic administration of the vehicle or CS-905 (Mean±S.E. %)

| Finding                        | Control 1 mg/kg/day | CS-905 1 mg/kg/day | CS-905 3 mg/kg/day |
|--------------------------------|---------------------|--------------------|--------------------|
| Intimal proliferation of artery | 34.1±8.8            | 26.6±9.1           | 5.4* ±0.8          |
| Glomerular sclerosis           | 42.5±5.6            | 40.4±8.4           | 22.3* ±3.9         |
| Pericapsular fibrous thickening| 46.3±5.7            | 28.2±9.2           | 11.8**±2.4         |

*P<0.05, **P<0.01, compared with the values in the control group.

### Table 4. Renal tubular injury in the SHR kidney after chronic administration of the vehicle or CS-905

| Finding          | Control (n=4) | 1 mg/kg/day (n=5) | CS-905 3 mg/kg/day (n=4) |
|------------------|---------------|-------------------|--------------------------|
| Epithelial necrosis| 2* 1 1        | 5                 | 4                        |
| Epithelial degeneration| 1 1 2        | 4 1               | 4                        |
| Dilatation       | 2 2           | 1 4               | 4                        |

-= None, ±: Very slight, +: Slight, ++: Moderate, +++: Severe. *: Number of animals.

### Table 5. UV, UNa+V, UK+V and UCl−V in the 0–6 hr, 6–24 hr and 0–24 hr period after acute administration of the vehicle or CS-905

| Dose (mg/kg) | Time (hr) | UV (ml/kg) | UNa+V (μEq/kg) | UK+V (μEq/kg) | UCl−V (μEq/kg) |
|--------------|-----------|------------|----------------|---------------|----------------|
| Control      | 0–6       | 16.4±1.3   | 1482±198       | 516±39        | 1420±178       |
|              | 6–24      | 13.8±1.8   | 2100±204       | 931±85        | 1784±218       |
|              | Total     | 30.3±2.6   | 3582±283       | 1447±117      | 3204±309       |
| CS-905 1     | 0–6       | 19.5±2.3   | 1988±248       | 587±71        | 1932±257       |
|              | 6–24      | 15.3±2.3   | 2171±245       | 1407±273      | 1508±175       |
|              | Total     | 34.7±3.8   | 4159±432       | 1993±330      | 3440±370       |
| CS-905 3     | 0–6       | 28.7±2.4** | 2765±391**     | 638±107       | 2447±359       |
|              | 6–24      | 18.1±3.4   | 1718±522       | 1366±185      | 1446±481       |
|              | Total     | 46.8±4.2** | 4483±372*      | 2004±286      | 3893±377       |

All values are the mean±S.E. of 8 rats. *P<0.05, **P<0.01, compared with the values in the control group.
were 0.93±0.05 and 1.14±0.06 U/kg/day, respectively, vs. 1.02±0.06 U/kg/day in the control SHR.

Discussion

Sodium retention is a major adverse effect of antihypertensive vasodilators such as hydralazine and minoxidil, which necessitates the concomitant use of diuretics in clinical situations (5, 6). On the other hand, a large body of evidence suggests that Ca++ antagonists cause a diuresis. For example, nitrendipine, nifedipine and diltiazem have been reported to produce natriuresis and diuresis in addition to hypotension following oral administration to SHR (7, 8). Though natriuretic and diuretic effects of Ca++ antagonists are beneficial characteristics as antihypertensive agents, the underlying mechanisms are poorly understood at present. A micropuncture study has shown that felodipine, a dihydropyridine Ca" blocker, inhibits sodium reabsorption in the proximal tubule (9). Chronic administrations of felodipine, however, do not cause natriuresis, probably because of compensatory mechanisms such as activation of the sympathetic nervous system and the renin-angiotensin system. In the present study, CS-905 caused natriuresis after chronic as well as acute administration. This difference between felodipine and CS-905 may be explained on the basis of the fact that CS-905 causes a relatively little compensatory increase of the renin-angiotensin system as evidenced in Fig. 5. The slowly developing antihypertensive action of CS-905 (4) may account for the lack of activation of the renin-angiotensin system.

It is known that the glomeruli of SHR are protected from the increased perfusion pressure by preglomerular vasoconstriction (10). The relative intrarenal vasoconstriction frequently noted in patients with benign essential hypertension (11) may correspond to the renal protective mechanism in SHR, a commonly studied animal model of human essential hypertension. Glomeruli of SHR are, therefore, protected from systemic hypertension by relative afferent arteriolar vasoconstriction. However, the medullary blood flow derived from deep cortical efferent arterioles is not autoregulated, but is increased when renal artery pressure increases (12, 13). Feld et al. (14) found that urinary protein excretion in SHR increased with age and that increased urinary protein at age 45 weeks was predominantly of the albumin fraction. Based on these observations and histological examination of the kidney, they concluded that juxtaglomerular glomeruli were the site of protein leakage. Although we did not analyze the fractions of protein in the urine in the present study, increased urinary protein observed in the control SHR was most probably of the albumin fraction. Indeed histological examination in the present study also revealed that glomeruli in the juxtamedullary zone suffered most severe injury.

The 15 week treatment with CS-905 starting from 23 weeks of age produced a significant decrease of urinary protein excretion: 39 and 48% reductions, respectively, for the 1 and 3 mg/kg/day groups as compared with the control SHR. Although we did not measure urinary protein excretion at the start of dosing, i.e., at 23 weeks old, the another series of experiments demonstrated that SHR at this age had already a higher urinary protein excretion compared to age-matched WKY (Fig. 1). These results suggest that CS-905 has a protective effect against the progression of renal injury in SHR.

If the site of protein leakage was the juxtaglomerular glomeruli, we have to consider two possible reasons for the protective action by CS-905. The first possibility is a secondary effect due to the antihypertensive action of CS-905. However, it has been observed that the triple drug therapy consisting of reserpine, hydralazine and hydrochlorothiazide produced a significant aggravation of glomerular lesions in SHR in spite of the reduction of blood pressure to normal levels (15). These findings suggest the decrease of urinary protein excretion produced by long-term administrations of CS-905 cannot solely be explained on the basis of sustained lowering of blood pressure.

Histological examination of the kidney demonstrated that intimal thickening of the arteries was reduced by chronic treatment with CS-905. In other experiments using the tracer microsphere technique, we have found that the agent increases renal blood flow in
conscious SHR (K. Oizumi, unpublished observation). These facts favor the notion that CS-905 produces a sustained improvement in the renal circulation. These renal hemodynamic changes together with sustained systemic hypotension may have protected the juxtamedullary glomeruli that are most vulnerable to hypertension.

N-Acetyl-β-glucosaminidase (NAG) is a hydrolytic enzyme that is localized in the lysosomal enzyme fraction of proximal renal tubular cells. Urinary NAG activity is considered to be a very sensitive index of renal tubular damages (16–18). In the present study, the urinary NAG activity of SHR was significantly higher than that of WKY at 17 weeks of age, when we started dosing of CS-905, suggesting that tubular injury had already been observed in SHR at 17 weeks of age. Histological findings in the kidney excised from the control SHR at the end of the dosing period also indicate the presence of tubular lesions, although they were less severe than glomerular damages. Long-term administration of CS-905 tended to decrease urinary NAG activity, whereas a single oral administration did not affect it at all. It is therefore possible that the 15 week treatment with CS-905 was not long enough to produce a significant decrease in urinary NAG activity. Alderman et al. (19) reported that one year-treatment with diuretics drugs and beta-adrenergic blockade in hypertension. A comparative study of minoxidil and hydralazine. Circulation 115, 571–582 (1972)

In conclusion, both acute and chronic administration of CS-905 increase urinary sodium and water excretion and only the chronic administration reduces urinary protein excretion. These effects of CS-905 on renal functions may be beneficial in antihypertensive therapy, although clarification of underlying mechanisms requires further studies such as examination of intrarenal hemodynamics and fractionation of urinary protein before and after chronic treatment with CS-905.

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