Effects of Dihydropyridine Ca Blockers on the Renal Function in Nephrotic Spontaneously Hypertensive Rat (SHR)

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An animal model having both hypertension and reduced renal function was produced by intraperitoneal injection of puromycin aminonucleoside (PAN) in spontaneously hypertensive rat (SHR). Using this model, two different dihydropyridine Ca blockers, CS-905 and nicardipine, were compared with regard to the relationship between hypotensive effects and changes in renal function in a conscious state. A single oral administration of CS-905 or nicardipine at doses of 3 or 10 mg/kg produced a dose-dependent decrease in blood pressure and an increase in heart rate. Glomerular filtration rate (GFR) was decreased only at 10 mg/kg. However, there was a substantial difference between the two drugs with respect to the relationship between blood pressure and GFR. The decrease of GFR by nicardipine was observed when blood pressure was at the lowest level, while GFR decreased by CS-905 returned to the initial level when blood pressure reached a nadir. Percent decrease of GFR by CS-905 was significantly less than that by nicardipine although both agents produced almost the same degree of peak hypotension. These results suggest the decrease in GFR by Ca blockers depends not only on the degree of hypotension but other factors as well, such as the rate of blood pressure lowering.

Despite the hypotension, both agents produced a marked natriuresis. Since the natriuresis was not accompanied by an increase in GFR, it was assumed that the natriuretic effect of Ca blockers stemmed from their tubular effects rather than glomerular ones.

Keywords: CS-905; nicardipine; spontaneously hypertensive rat (SHR); puromycin aminonucleoside; glomerular filtration rate (GFR); natriuresis

In normal kidneys, where the renal autoregulation system functions properly, renal blood flow (RBF) and glomerular filtration rate (GFR) are preserved despite the change in perfusion pressure. However, RBF and GFR in diseased kidneys are likely to be affected by the change in perfusion pressure due to a possible defect in the autoregulation system.1,2) Thus, excessive antihypertensive therapy in hypertensive patients with diseased kidneys could produce a drop in renal perfusion pressure leading to a reduction in renal function.3) In fact, Diamond et al. reported that nifedipine, a Ca blocker, produced significant renal dysfunction in patients with decreased GFR.4) However, relatively little information is available about the relationship between blood pressure and renal function in experimental renal diseases.

It is well known that puromycin aminonucleoside (PAN) induces a nephrotic syndrome characterized by proteinuria and reduced renal function in rats.5,6) In the present study, we produced an animal model having both hypertension and reduced renal function by injecting PAN to spontaneously hypertensive rat (SHR). Using this model, we compared the effects on blood pressure and renal function after a single oral administration of two different dihydropyridine Ca blockers: nicardipine with fast onset and short duration of action, and CS-905 with slow onset and long duration of action7); (±)-3-(1-diphenylmethyl-azetidin-3-yl)-5-isopropyl-2-amino-1,4-dihydropyridine-2,5-pyridine-3,5-carboxylate.

MATERIALS AND METHODS

Male 1 week-old SHR obtained from Hoshino Laboratory Animals (Japan) were used. Four or 5 weeks after intraperitoneal injection of PAN (150 mg/kg), the animals were placed in individual metabolic cages and the spontaneous urine was sampled for 0–24 h under a food deprived condition. Rats whose urinary protein excretion (Upro.) exceeded 400 mg/24 h were used in this experiment. Urinary protein was determined with a kit purchased from Wako (Micro TP-test).

Surgical Preparation Animals were anesthetized with sodium pentobarbital, 40 mg/kg i.p. The femoral artery and vein were catheterized with polyethylene cannulae (PE10). The other ends of both cannulae were led under the skin and exteriorized at the back of the neck. A bladder catheter made of stainless steel was placed according to the method of Gellai and Valtin.8) Animals were allowed to recover for 2–3 d.

Effects of Ca Blockers on Renal Function Experiments were performed with animals placed in a Bollman cage in a conscious state. The aortic cannula was connected to a pressure transducer (Nihon Kohden, TP-200T), and blood pressure and heart rate (HR) were continuously recorded on a pen-writing oscilloscope (Nihon Kohden, RJG-4128). Saline containing 4% inulin was infused through the venous cannula at a rate of 8 μl/100 g/min throughout the experimental period. After an equilibration period of 180 min, urine was collected for 60 min via the bladder catheter and a midpoint blood sample (150 μl) was obtained via the aortic cannula.

CS-905 and nicardipine were dissolved in Tween 80 and in dimethyl sulfoxide (DMSO), respectively, followed by dilution with distilled water (1: 4). CS-905, nicardipine and the vehicles for each agent were administered by gavage in a volume of 0.2 ml/100 g. To avoid the artifact due to stress caused by oral administration, a 10 min equilibration period was allowed before the urine collection was started. Urine was sampled for the following 4 periods: 10–60 min,
1—2 h, 2—4 h and 4—5 h. Blood sampling was performed at the midpoint of each period.

After the blood was centrifuged, plasma was stored for inulin measurement. Inulin was determined by the anthrone method\(^a\) and urinary Na was measured with a flame photometer (Hitachi 750). GFR was measured as inulin clearance.

All values were expressed as the mean ± S.E. Statistical analysis between the values of PAN-treated SHR and those of normal SHR was performed using Student's t-test. Statistical significances among 3 groups were assessed by Duncan's test. In order to compare the decrease of GFR after treatment with CS-905 and nicardipine both at the dose of 10 mg/kg p.o., percent decrease from the pre-administration value was calculated and analyzed using Student’s t-test. Statistical significance was set at the level of \(p<0.05\).

RESULTS

Table I shows the control values for body weight (BW), mean blood pressure (MBP), Upro, and GFR in the PAN-treated SHR and the control SHR. BW of the PAN-treated SHR was lower than the control SHR, while the MBP of the two groups was similar. PAN-treated SHR showed a marked proteinuria and about a 43% reduction in GFR compared to that of the control SHR.

Figures 1A and B show the time course for MBP and HR after a single oral administration of CS-905 and nicardipine, respectively. Both agents lowered blood pressure in a dose-dependent manner. There were no changes in blood pressure or HR in the vehicle treated groups except for a sharp increase in the latter seen immediately after administration, presumably due to stress from gavage administration. CS-905 produced a gradual decrease in MBP which reached a peak after 2 h and stayed at this level during the experimental period. The HR peaked at 1—2 h after CS-905. With nicardipine, blood pressure reached a nadir 15 min after administration and returned near pre-administration values in 2 to 5 h. Maximal increase of HR was also observed 15 min after nicardipine administration. The peak reduction of blood pressure following a single oral administration of CS-905 10 mg/kg (−63 mmHg at 3—5 h) was similar to that following nicardipine 10 mg/kg (−67 mmHg at 15 min).

The changes in GFR after an oral administration of

TABLE I. BW, MBP, Upro, and GFR in PAN-Treated SHR and Control SHR

<table>
<thead>
<tr>
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<th>BW (g)</th>
<th>MBP (mmHg)</th>
<th>Upro. (mg/kg/d)</th>
<th>GFR (μl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SHR</td>
<td>361 ± 9</td>
<td>172 ± 6</td>
<td>30 ± 2</td>
<td>3882 ± 203</td>
</tr>
<tr>
<td>PAN treated SHR</td>
<td>299 ± 8(^a)</td>
<td>168 ± 3</td>
<td>729 ± 73(^b)</td>
<td>2194 ± 128(^b)</td>
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All values are the mean ± S.E. from 7 SHR and 16 PAN-treated SHR.
\(a\) \(p<0.001\), compared with the values in SHR.

Fig. 1. Time Courses for MBP and Percent Changes in HR after a Single Oral Administration of CS-905 (A) or Nicardipine (B) in Conscious Rats

A: —, vehicle; —, CS-905 3 mg/kg; —, CS-905 10 mg/kg. B: —, vehicle; —, nicardipine 3 mg/kg; —, nicardipine 10 mg/kg. All values are the mean ± S.E. from 5 rats.
CS-905 or nicardipine are shown in Figs. 2A, B. Both agents at a dose of 10 mg/kg significantly lowered GFR at the different time periods; 1—2 h for CS-905 and 10—60 min for nicardipine. At these periods, heart rate increased maximally and blood pressure decreased at the greatest rate, suggesting that the baroreceptor reflex was maximally activated. The increase of HR was much less with CS-905 (27 ± 5%) than with nicardipine (40 ± 4%). The decrease of GFR with CS-905 (32 ± 8%) was also significantly less than that with nicardipine (61 ± 7%).

As shown in Fig. 3, nicardipine produced a significant decrease in Na excretion during the 10—60 min period, when GFR decreased maximally. Marked natriuresis occurred at 2—5 h after CS-905 at 3 mg/kg and 2—4 h after nicardipine at 10 mg/kg. Nicardipine at 3 mg/kg and CS-905 at 10 mg/kg had no effect on urinary Na excretion.

DISCUSSION

Excessive hypotension below the range of renal auto-regulation leads to a reduction in RBF and GFR. In the present study, CS-905 and nicardipine, both at a dose of 10 mg/kg decreased GFR (Fig. 2). However, there was a marked difference between the two drugs with respect to the relationship between the changes in blood pressure and GFR. Nicardipine reduced GFR when blood pressure was at the lowest level (Fig. 1B). On the other hand, GFR was decreased by CS-905 before blood pressure reached a nadir and it returned to the initial level when blood pressure was at the lowest level (Figs. 1A, 2A). These results suggest that the reduction in GFR by Ca blockers depends not only on the degree of hypotension but on other factors, although the range of renal autoregulation in this model is not determined.

A fall in blood pressure by Ca blockers stimulates the sympathetic nerve system and the renin–angiotensin system via the baroreceptor reflexes. Under this circumstance, elevations of norepinephrine and angiotensin II concentrations reduce GFR, because these
hormones constrict the afferent and efferent arterioles and the mesangial cells. Thus the pressor system activated by antihypertensive drugs may limit their blood pressure–lowering effect at the cost of decreased RBF and GFR. This notion is supported by the fact that both nicardipine and CS-905 decreased GFR when HR was markedly increased (Figs. 1AB, 2AB).

Although the peak reduction in blood pressure by CS-905 at 10 mg/kg p.o. was almost the same as that by nicardipine at the same dose, the increase in HR by CS-905 was less than that by nicardipine (Fig. 1). With regard to this difference, it is known that the intensity of baroreceptor reflexes is determined not only by the degree of hypotension but by the rate of blood pressure fall. In this study, CS-905 lowered blood pressure at a slower rate than nicardipine. These findings taken together suggest that CS-905 triggered the baroreceptor reflex less intensely than nicardipine did. We previously reported that nicardipine produced much greater increase of plasma renin activity (PRA) than CS-905 did, whereas the degree of hypotension induced by the two agents was almost the same in conscious perinephritic hypertensive dogs. These results may account for why the decrease of GFR with CS-905 was less than that with nicardipine.

Thus the results of the present study, together with previous findings, suggest that CS-905 has a less negative effect on renal function than nicardipine. These characteristics of CS-905 would provide a potential advantage in treating hypertensive patients with decreased renal function. Since it is widely acknowledged that many hypertensive patients have reduced renal function as a complication, CS-905 could become a useful therapeutic agent in antihypertensive therapy.

CS-905 at 3 mg/kg p.o. and nicardipine at 10 mg/kg p.o. produced a marked natriuresis without increasing GFR during a 2–5 h period and a 2–4 h period, respectively (Fig. 3). It is well known that dihydropyridine Ca blockers have a natriuretic effect; however, it is controversial whether or not the natriuresis due to the Ca blockers is accompanied by an increase in GFR. The present results clearly demonstrate that the Ca blocker can cause natriuresis without an increase in GFR. It is conceivable that Ca blockers cause natriuresis primarily via their tubular effects. CS-905 at a higher dose, 10 mg/kg, did not produce natriuresis. A possible explanation for this lack is that the natriuresis due to tubular effects is counteracted by the reduction of renal perfusion pressure due to excess hypotension.

REFERENCES