Effects of a New Dihydropyridine Derivative, FRC-8653, on Blood Pressure in Conscious Spontaneously Hypertensive Rats

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Antihypertensive effects of FRC-8653, a new 1,4-dihydropyridine derivative, and its combined effects with an angiotensin converting enzyme (ACE) inhibitor, a diuretic, and a β -adrenergic blocking agent were examined in conscious spontaneously hypertensive rats (SHR). When administered intravenously to SHR (10, 30 μ g/kg), FRC-8653 lowered blood pressure more slowly and sustained it longer than nifedipine and nicardipine. Consecutive once-daily administrations of FRC-8653 to SHR (3 mg/kg, *p.o.*) produced a stable reduction of blood pressure throughout the experimental period of 29 d. When blood pressure was continuously measured for 24 h in conscious unrestricted SHR, orally administered FRC-8653 produced a long-lasting reduction in blood pressure. When concomitantly used with atenolol (30 mg/kg, *p.o.*), the antihypertensive effect of FRC-8653 was augmented in both potency and duration. However, simultaneous administration of captopril (30 mg/kg, *p.o.*) or hydrochlorothiazide (2.5 mg/kg, *p.o.*) did not modify the antihypertensive effect of FRC-8653.

Keywords — FRC-8653; SHR; hypertension; calcium antagonist

Introduction

It has been widely accepted that compounds having a 1,4-dihydropyridine (DHP) structure, including nifedipine and nicardipine, have a vasodilating effect due to calcium antagonistic action and that they are useful as antihypertensive drugs.¹⁾ FRC-8653 is a new DHP derivative synthesized by Fujirebio Inc. (Tokyo). This compound is confirmed to have calcium antagonistic action, which seems to account for its antihypertensive effect.^{2,3)} In various experimentally hypertensive rats, orally administered FRC-8653 has shown a gradual antihypertensive action with a duration of more than 7 h.⁴⁾ Thus the antihypertensive effect of FRC-8653 is slow in onset and long-lasting. In the present study in SHR, the authors compared the onset and duration of the antihypertensive activities of FRC-8653, nifedipine and nicardipine by administering these drugs intravenously to circumvent the problem of absorption from the digestive tract. Furthermore, repeated oral administration of FRC-8653 was made in SHR for 29 d, to examine the tolerability of its anti-

hypertensive action.

Clinically, calcium antagonists are often used in combination with other types of antihypertensive drugs. Therefore, it is necessary to investigate the efficacy and the duration of the antihypertensive effects of FRC-8653 in concomitant usage with other antihypertensive drugs. In the present study, the antihypertensive effects of FRC-8653 combined with captopril (an ACE inhibitor), hydrochlorothiazide (a diuretic), and atenolol (a β -blocker) were continuously examined for 24 h in conscious unrestricted SHR.

Materials and Methods

Animals — Five- to 7-week-old male SHR were purchased from Charles River Japan (Kanagawa, Japan) and maintained in our animal house in a specific pathogen-free state (temperature: 23 ± 3 °C; humidity: 55 ± 15 %; lighting: 7:00 a.m.—7:00 p.m.). Standard laboratory animal chow containing 0.28% of sodium (CRF-1, Charles River Japan) and tap water were provided ad libitum. Sixteen to 20

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weeks old animals were used in all experiments.

Blood Pressure Measurements — In the experiments using intravenous administration, SHR were anesthetized with ether and the right femoral artery and vein were cannulated. The venous cannula was filled with physiological saline, and that of the artery with physiological saline containing 100 unit/ml heparin (Kodama Co., Tokyo). Before recovering from the anesthesia the rats were placed in a sling suit (Alice King Chatham, CA, U.S.A.) and half-restricted to a sling frame. For the measurement of mean arterial blood pressure, a pressure transducer (Nihon Kohden SCK-580, Tokyo) combined to a carrier amplifier (Nihon Kohden AP-601G) was connected to the arterial cannula, and heart rate was also measured using a cardiometer (Nihon Kohden AT-601G) by counting the pulse from the blood pressure recordings. These parameters were recorded continuously on a ink-writing oscillograph (Nihon Kohden WT-687G). After the rats had totally awakened and were accustomed to the environment, the drugs were cumulatively administered through the venous cannula.

In the experiment of repeated consecutive administration, FRC-8653 (3 mg/kg, *p.o.*) was administered to SHR once daily for 29 d, and systolic blood pressure and heart rate were plethysmographically⁵⁾ monitored before and 3 h after drug administration at one-week intervals.

The antihypertensive effects of FRC-8653 with concomitant administration of other antihypertensive drugs were investigated as follows.

Polyethylene tubing (Clay Adams PE-50, NJ, U.S.A.) was inserted retrogressively into the carotid artery of SHR under anesthesia with sodium pentobarbital (70 mg/kg, *i.p.*). The other end of the tubule was passed subcutaneously to the dorsal side of the neck and led to the outside of the body. Rats were kept individually in cages under the conditions in the animal house mentioned above and were used for the experiments after postoperative recovery for 3–4 d. The measurement of blood pressure and of heart rate was started at 9:00 a.m. and was made continuously for 24 h under a conscious and unrestricted condition with the same system as the intravenous administration experiment. After habituation in the observation cages for 1 h, drugs were administered to rats at 10:00 a.m. After being dissolved in vehicle, polyethylene glycol 400-R (Nippon Oil and Fats Co., Tokyo), FRC-8653 was administered at the dose of 3 mg/kg, *p.o.* (1 ml/kg). In the combination therapy, the other antihypertensive drugs, captopril (30 mg/kg, *p.o.*), hydrochlorothiazide (2.5 mg/kg, *p.o.*) and atenolol (30 mg/kg, *p.o.*) were concomitantly given with FRC-8653. Rats were fasted and tap water was given *ad libitum* throughout the day of measurement. Mean arterial blood pressure was monitored using a polygraph system, which data were stored in a computer system (Nippon Electric Company PC-9801, Tokyo) at 3 s intervals. These data, averaged every 5 min, were used as mean arterial blood pressure at each time after drug administration. The initial values were defined as the mean of the data obtained during the 0.5 h be-

TABLE I. Effects of Intravenous Administration of FRC-8653, Nifedipine and Nicardipine on the Mean Blood Pressure in Conscious, Spontaneously Hypertensive Rats

Drugs	Dose ($\mu\text{g/kg}$)	Blood pressure ^{a)}			
		Initial	Maximum decrease	Peak time ^{b)}	Duration ⁵⁰ ^{c)}
FRC-8653	10	151.2 ± 3.8	-24.5 ± 3.2	1.1 ± 0.3	7.2 ± 1.0
	30		-43.5 ± 2.1	1.7 ± 0.1	20.4 ± 4.1
Nifedipine	30	150.5 ± 4.3	-26.3 ± 1.5	0.6 ± 0.1	5.4 ± 0.7
	100		-44.3 ± 1.7	0.6 ± 0.1	7.5 ± 0.9
Nicardipine	10	145.2 ± 4.0	-23.0 ± 2.1	0.6 ± 0.1	2.5 ± 0.5
	30		-51.5 ± 3.2	0.8 ± 0.1	6.8 ± 1.4

a) Each value represents the mean $\pm$ S.E. of 6 experiments. b) Time required to achieve the maximum decrease of blood pressure. c) Time required for the recovery to half of the maximum decrease.

Fig. 1. Changes in Systolic Blood Pressure (SBP) and Heart Rate (HR) of SHR with the Repeated Daily Oral Administration of FRC-8653

Three mg/kg of FRC-8653 (●) or 5% gum Arabic solution (○) as control was given. Indirect measurements of blood pressure were made every week before and 3 h after dosing a) $p < 0.01$ vs. control.

fore administration, and these were not significantly different among the experimental groups. Each group consisted of 6 animals, which were randomly divided.

Drugs — FRC-8653 and captopril were synthesized at Ajinomoto Co., Inc. Nicardipine was synthesized at Fujirebio Inc. Nifedipine, hydrochlorothiazide, and atenolol were purchased from Sigma Co., MO, U.S.A.

Statistical Analysis — The results were expressed as the mean values $\pm$ standard errors. Statistical analysis was performed using Student's *t*-test, and a *p* value of less than 5% was considered significant.

Results

Antihypertensive Effects of Intravenous Administration in Conscious SHR

As shown in Table I, intravenous administration of FRC-8653, nifedipine, and nicardipine to conscious SHR lowered blood pressure dose-dependently. The doses to obtain a decrease of about 25 mmHg in mean blood pressure were 10 μ g/kg for FRC-8653 and nicardipine, and 30 μ g/kg for nifedipine. Likewise, the doses required to lower blood pressure about 45 mmHg were 30 μ g/kg for FRC-8653, 30 μ g/kg for nicardipine and 100 μ g/kg for nifedipine. Among

Fig. 2. Changes in Mean Arterial Blood Pressure (MBP) after the Oral Administration of FRC-8653

Drug was dissolved in PEG-400R and given to the animal (3 mg/kg, *p.o.*). In the control group, PEG-400R was given at the same amount as that of drug solution (1 ml/kg, *p.o.*). Each point represents the mean $\pm$ S.E. value of blood pressure from 6 animals. a) control, b) FRC-8653 (3 mg/kg).

the three drugs tested, FRC-8653 had the slowest onset of decreasing blood pressure, and it maintained its reduction of blood pressure the longest.

Antihypertensive Effects of the Repeated Oral Administrations of FRC-8653 in SHR

Changes in blood pressure and heart rate produced by repeated administration of FRC-8653 to SHR (3 mg/kg, *p.o.*) are shown in Fig. 1. A decrease in blood pressure but no significant change in heart rate was observed 3 h after the first administration. The pre-dosing value on each day (the value 24 h after the latest administration) was not significantly different from that before the first administration.

Antihypertensive Effects of FRC-8653 Concomitantly Given with Other Antihypertensive Agents in Conscious SHR

Decreases in blood pressure induced by the

simultaneous oral administration of FRC-8653 and captopril to SHR were compared with those brought about by the separate administration of each agent. After the administration of FRC-8653 (3 mg/kg, *p.o.*), the blood pressure remained at a decreased level until 12 h, then it gradually returned to the predosing level at 24 h (Fig. 2a and 2b). A statistically significant fall in blood pressure vs. the control group was observed until 7 h. Thirty mg/kg of captopril produced a slight but long-lasting decrease in blood pressure, which was significant vs. the control group until 4 h (Fig. 2a and 3a). The concomitant oral administration of captopril did not change the chronogram of the effect of FRC-8653 (Fig. 3b vs. 2b). No difference in heart rate was observed between concomitant use and single use of FRC-8653 throughout the ex-

Fig. 3. Changes in Mean Arterial Blood Pressure (MBP) after the Administration of (a) Captopril (30 mg/kg, *p.o.*) or after the Concomitant Oral Administration of (b) FRC-8653 (3 mg/kg, *p.o.*) and Captopril (30 mg/kg, *p.o.*)

Each drug was dissolved in PEG-400R and given to the animal (1 ml/kg, *p.o.*). Each point represents the mean $\pm$ S.E. value of blood pressure from 6 animals.

Fig. 4. Changes in Mean Arterial Blood Pressure (MBP) after the Administration of (a) Hydrochlorothiazide (2.5 mg/kg, *p.o.*), or after the Concomitant Oral Administration of (b) FRC-8653 (3 mg/kg, *p.o.*) and Hydrochlorothiazide (2.5 mg/kg, *p.o.*)

Drugs were dissolved in PEG-400R and given to the animal (1 ml/kg, *p.o.*). Each point represents the mean $\pm$ S.E. value of blood pressure from 6 animals.

Fig. 5. Changes in Mean Arterial Blood Pressure (MBP) and Heart Rate (HR) after the Concomitant Oral Administration of FRC-8653 (3 mg/kg, *p.o.*) and Atenolol (30 mg/kg, *p.o.*), or after the Separate Administration of Each Drug a) control, b) FRC-8653 (3 mg/kg), c) atenolol (30 mg/kg), d) FRC-8653 (3 mg/kg) + atenolol (30 mg/kg).

Drugs were dissolved in PEG-400R and given to the animal (1 ml/kg, *p.o.*). In the control group, PEG-400R was given at the same amount as that of drug solution. Each point represents the mean $\pm$ S.E. value of MBP or HR from 6 animals. a) $p < 0.05$ vs. FRC-8653 (3 mg/kg, *p.o.*).

perimental period (data not shown).

The antihypertensive effects of concomitant oral administration of FRC-8653 and hydrochlorothiazide were investigated (Fig. 4a and 4b). Hydrochlorothiazide (2.5 mg/kg, *p.o.*) tended to lower the blood pressure slightly compared to the control level, though without statistical significance vs. the control (Fig. 2a and 4a). The antihypertensive effect of FRC-8653 (3 mg/kg, *p.o.*) given concomitantly with hydrochlorothiazide (2.5 mg/kg, *p.o.*) was not different from that of FRC-8653 alone (Fig. 2b and 4b). No change was observed in heart rate between the single use of FRC-8653 and the concomitant use (data not shown).

Changes in blood pressure and heart rate after the simultaneous oral administration of FRC-8653 and atenolol were compared with those after the separate administration of each agent (Fig. 5a—d). When atenolol was given at a dose of 30 mg/kg, *p.o.*, a decrease in blood pressure began 2 h after the administration, and a stable decrease in blood pressure by about 30 mmHg was observed from 4 to 12 h. (Fig. 5c). The fall was statistically significant from 5 h to 10 h vs. the control group (Fig. 5a and 5c). A decrease in heart rate was also observed. With the concomitant administration of FRC-8653 and atenolol, the decrease in blood pressure was significantly greater than that produced by FRC-8653 alone. Furthermore, the duration of the effect had a tendency to be prolonged compared to the separate administration of each agent (Fig. 5b, 5c, and 5d). A decrease in heart rate, which was induced by atenolol alone, was similarly observed with the combined administration of FRC-8653 and atenolol (Fig. 5c and 5d).

Discussion

In the present experiments, the decrease in blood pressure produced by intravenous administration of FRC-8653 was found to be equal to that brought about by nifedipine, and to be greater than that induced by nifedipine. Furthermore, its onset was slow and the duration was long-lasting. These results indicate that the slow appearance and long duration of the antihyper-

tensive action of orally administered FRC-8653⁴⁾ are not simply due to slow absorption of the drug from the digestive tract. The *in vivo* long-acting nature of FRC-8653 may be partially explained by *in vitro* studies that have shown that the calcium antagonistic actions of FRC-8653 are slow in onset and long-lasting in duration.^{2,3)}

In the present experiment, when FRC-8653 was orally given consecutively to SHR for 29 d, there was neither a diminution nor an enhancement of its antihypertensive action. The reason repeated administration of FRC-8653 does not induce tolerance despite its vasodilating action may be attributed to its natriuretic action (Hosono *et al.* unpublished observation). We cannot evaluate whether the chronogram changed after the repeated oral administration of FRC-8653 from this study, and this remains to be analyzed in the future.

Clinically, stepwise therapy is adopted in the treatment of essential hypertension; diuretics, β -adrenergic blocking agents, and ACE inhibitors as well as calcium antagonists are proposed as the first-step drugs. Further, in the second step the combined use of these agents is proposed.⁶⁾ Therefore, we examined the effects of combined administration of FRC-8653 and other drugs that may be used concomitantly with FRC-8653. Continuous measurement of the blood pressure in SHR for 24 h would be important to evaluate a reduction of blood pressure and its duration in the concomitant administration of FRC-8653 and other antihypertensive drugs. In the present study, direct blood pressure measurement in conscious unrestricted SHR was performed in order to examine the combined effects of FRC-8653 and other antihypertensive agents on both the extent and duration of antihypertensive efficacy.

In the concomitant administration of FRC-8653 and captopril, an ACE inhibitor, the antihypertensive effect was not significantly potentiated in comparison with FRC-8653 alone. Reportedly, blood renin activity in SHR is within or slightly below the range of normotensive rats,⁷⁻⁹⁾ although Asaad and Antonaccio have reported enhanced renin activity at the blood vessel wall from SHR.¹⁰⁾ Therefore the contribu-

tion of the renin-angiotensin system to the maintenance of hypertension seems to be small in SHR compared to that in renovascular hypertensive models. This might explain the lack of increase with the concomitant use of captopril and FRC-8653. Similar results to the present study have been reported using other DHP calcium antagonists.¹¹⁾ In any case, plasma renin activity should be measured in our experimental model to precisely evaluate the effect of FRC-8653 on a blood pressure control mechanism maintained by the renin-angiotensin system.

In the combined use of FRC-8653 and hydrochlorothiazide, a diuretic, both the extent and duration of antihypertensive action were about the same as those obtained by FRC-8653 alone. This result was in agreement with clinical reports that the combined use of a DHP-type calcium antagonist and a diuretic agent did not produce a synergistic effect in the antihypertensive action.^{12,13)}

In the present experiment, we used atenolol as a representative β -blocking agent, taking its clear antihypertensive effect in SHR reported in previous papers into consideration.^{14,15)} When FRC-8653 and a β_1 -selective blocking agent, atenolol, were given concomitantly, the antihypertensive action tended to be enhanced compared to that produced by each agent given separately. An increase in heart rate was not observed in the concomitant usage. This may be explained in that the tachycardia induced by vasodilating drugs is diminished by the blockade of sympathetic nerves regulating cardiac function by the β -adrenergic blocking agent. Our result was compatible with clinical reports that the combined use of a β -adrenergic blocking agent and a calcium antagonist produces an augmented antihypertensive effect and diminishes the tachycardia induced by the calcium antagonist.¹⁶⁾

The effect of a calcium antagonist on the circadian blood pressure and heart rate is a subject to be studied in detail. However, we should not argue this point from the results presented here, because a recent report proposes that continuous monitoring of blood pressure and heart rate for more than 4 d in a conscious unrestricted state is required to record a precise circadian blood

pressure in SHR.¹⁷⁾ This issue remains to be studied in the future.

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