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Effects of Long-term Oral Administration of NZ-105, a Novel Calcium Antagonist, With or Without Propranolol in Spontaneously Hypertensive Rats

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Abstract—A new calcium antagonist, NZ-105 ((±)-2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitrophenyl)-3-pyridinecarboxylate hydrochloride ethanol) (10 mg kg^{-1}, p.o.), showed slow-onset hypotensive effect in spontaneously hypertensive rats (SHRs). The tachycardia evoked by NZ-105 was completely prevented when combined with a β-adrenoceptor blocker, propranolol (20 mg kg^{-1}), which did not affect the hypotensive response to NZ-105. In long-term administration experiments for 12 weeks with SHRs, the systolic blood pressure in the control group increased with age and the heart rate was stable throughout the period. NZ-105 (10 mg kg^{-1} day^{-1}) alone and its combined treatment with propranolol (20 mg kg^{-1} day^{-1}) maintained the systolic blood pressure and heart rate at a low level compared with the control group. The hypotensive action of NZ-105 was reproducible after repeated dosing for 12 weeks. Long-term administration of propranolol affected neither the elevation of the systolic blood pressure nor the heart rate substantially. The heart weight per body weight was significantly reduced after the chronic combination of both drugs, suggesting that the cardiac hypertrophy accompanying hypertension was prevented.

NZ-105 ((±)-2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitrophenyl)-3-pyridinecarboxylate hydrochloride ethanol) is a newly synthesized 1,4-dihydropyridine class of calcium antagonist. This drug caused a slow-onset and long-lasting hypotensive effect in hypertensive model animals (Masuda et al 1990; Sakai et al 1991). NZ-105 is a calcium antagonist as shown by binding to 1,4-dihydropyridine receptors (Masuda et al 1991; Yamashita et al 1991), ^{45}Ca fluxes (Yamashita et al 1991) and electrophysiological studies (Tamura et al 1991).

Recently, calcium antagonists have been used as the first-choice drugs in the stepped-care approach to hypertension (US Joint National Committee 1988; Zanchetti et al 1989). Therefore, these drugs are required to have stable hypotensive effects in long-term use. Antihypertensive monotherapy sometimes fails to control blood pressure and these drugs are often used in combination to manage hypertension effectively. The dihydropyridine class of calcium antagonists sometimes induce adverse effects, such as palpitation, and are often used in combination with β-adrenoceptor blockers. In the present study, we investigated the antihypertensive effect of NZ-105 and its combination with propranolol, one of the β-adrenoceptor blockers widely used clinically in spontaneously hypertensive rats (SHRs).

Materials and Methods

Hypotensive effects after single administration

Male SHRs (Charles River Japan) were obtained at 12 weeks old (245–275 g) and divided into 3 groups (n = 5) as follows: NZ-105 (10 mg kg^{-1}) only; propranolol (20 mg kg^{-1}) only; both drugs in combination. The systolic blood pressure and heart rate were measured by the tail-cuff method (KN-210-1, Natsume, Osaka, Japan) before and 1, 3, 5, 7, 9 and 24 h after the oral administration of the drugs. The SHRs were prewarmed at 50°C for 3–5 min in a warm box and gently placed in a restraining cage on a heating plate (37°C); they were calmed for 5–15 min before the measurements.

Antihypertensive effects during long-term administration

Male SHRs were obtained at 8 weeks old (195–235 g) and divided into 4 groups (n = 6–7): vehicle (control); NZ-105 (10 mg kg^{-1}) only; propranolol (20 mg kg^{-1}) only; and NZ-105 (10 mg kg^{-1}) and propranolol (20 mg kg^{-1}) given in combination. The drugs were given orally once a day (1000–1100 h) for 12 weeks. The systolic blood pressure and heart rate were measured immediately before the daily administration, before and 2, 6, 8, 10, and 12 weeks after the start of drug administration. The hypotensive effects at 3 h after each daily administration were examined on the 1st day and at the 6th and 12th week; blood pressure and heart rate were measured as described above.

Determination of plasma renin activity and plasma aldosterone concentration after long-term administration

SHRs fasted for 12 h were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and 4 mL blood samples were obtained from the vena cava at 24 h after the last administration. Plasma renin activity and plasma aldosterone were determined by radioimmunoassay (SRL Co. Ltd, Tokyo, Japan).

Organ weights

SHRs fasted for 12 h were killed at 24 h after the last

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administration. The heart, liver and both kidneys were removed and weighed.

Drugs

NZ-105 (Central Research Laboratories of Nissan Chemical Industries Ltd, Chiba, Japan) and (±)-propranolol hydrochloride (Sigma Chemical Co. Ltd, St Louis, MO, USA) were dissolved or suspended in 0·5% methyl cellulose solution (SM 400, Shin-etsu Chemical Co., Tokyo, Japan) and given orally (2 mL kg^{-1}) through a gastric tube.

Statistical analysis

Statistical analysis was performed by Student's paired *t*-test or one-way analysis of variance followed by Dunnett's multiple comparison test.

Results

Hypotensive effects after single administration

Fig. 1 shows the time course of the effects of NZ-105, propranolol and both drugs combined on systolic blood pressure and heart rate in SHRs. NZ-105 (5, 10, 20 mg kg^{-1}, p.o.) produced a dose-dependent hypotensive action in SHR and the dose of 10 mg kg^{-1} was thought as the most adequate of the three doses (Masuda et al 1990). In this study, at 10 mg kg^{-1}, the hypotensive action of NZ-105 was slow in onset and reached its maximum at 3 h (−65·6±7·4 mmHg). Twenty-four hours after the administration, the blood pressure recovered to the basal level. The heart rate was increased by the hypotensive action of NZ-105.

Propranolol (20 mg kg^{-1}) alone did not produce significant changes in blood pressure, but the heart rate was significantly reduced. An increase in heart rate was completely prevented by the combination with propranolol, and the hypotensive effect of NZ-105 (at 3 h; −66·2±7·5 mmHg) was about the same as that of NZ-105 alone.

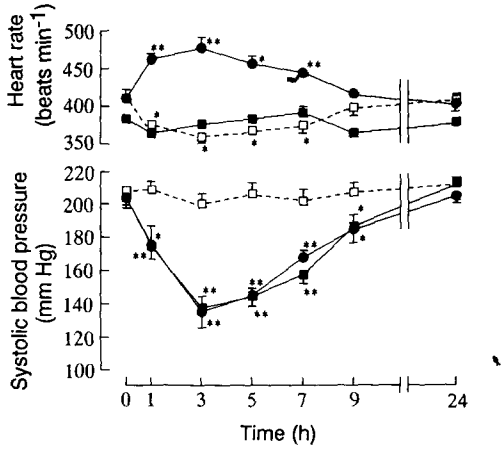

FIG. 1. Time course of the effects of a single administration of NZ-105 with or without propranolol on systolic blood pressure and heart rate in SHRs. Each drug was given orally; the drug and dose are as follows: ●——● NZ-105 alone (10 mg kg^{-1}); □----□ propranolol alone (20 mg kg^{-1}); ■——■ NZ-105 in combination with propranolol. Each point represents the mean±s.e. from 5 animals. $*P<0·05$, $**P<0·01$ compared with the pre-dose value.

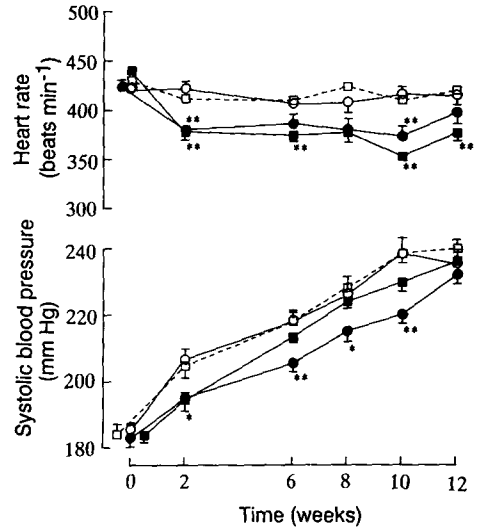

FIG. 2. Effects of long-term treatment with NZ-105, propranolol and both drugs on systolic blood pressure and heart rate in SHR. Each drug was given orally for 12 weeks to SHRs once a day starting at 8 weeks of age. The drug and dose were as follows: ○——○, control (0·5% methyl cellulose); ●——● NZ-105 alone (10 mg kg^{-1}); □----□ propranolol alone (20 mg kg^{-1}); ■——■ combination of NZ-105 with propranolol. Each point represents the mean±s.e. from 6 to 7 animals. $*P<0·05$, $**P<0·01$ compared with corresponding control values.

Antihypertensive effects during long-term administration

Fig. 2 shows the changes in systolic blood pressure and heart rate during 12 weeks of administration. In the control group, the blood pressure at the start of administration was 185·3±2·3 mmHg and increased with age reaching 235·4±4·0 mmHg at the 12th week. By repeated administration of NZ-105 (10 mg kg^{-1}), it was maintained at significantly low levels ($P<0·05$) compared with the control group at the 6th, 8th and 10th weeks. Propranolol (20 mg kg^{-1}) did not modify the increase in blood pressure, whereas, its combination with NZ-105 significantly suppressed ($P<0·05$) the blood pressure increase at the 2nd week as compared with the corresponding control value. Throughout the 12 weeks of the administration period, control and propranolol groups had stable heart rate, but in NZ-105 groups, the heart rate was lowered below the value at the start of the administration. Moreover, the heart rate in the combination group was significantly lower than in the control group, except for that at the 8th week (Fig. 2).

The body weight gain and the water and diet intake were not different in any of the groups (data not shown).

Hypotensive effects during long-term administration

On the 1st day and at the 6th and 12th weeks, the systolic blood pressure and heart rate were measured 3 h after the dosing, the time when the peak effect of NZ-105 was obtained. The blood pressure in control and propranolol groups did not change in comparison with the pre-dosing value. However, both in NZ-105 and combination groups, about the same degree of hypotensive effects were produced on the three days examined, indicating that tolerance did not develop in either group during the period of long-term

Table 1. Effects of NZ-105, propranolol and their combination on systolic blood pressure (SBP) and heart rate (HR) in spontaneously hypertensive rats.

Drug (dose)		1st day			6th week			12th week		
		Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change
Control	SBP	185·3±2·3	181·3±2·0	−2·1±1·5	218·3±2·4	211·0±4·5	−3·3±1·8	235·4±4·0	238·0±1·7	1·3±2·1
	HR	420·6±6·9	421·4±3·7	0·3±1·2	405·7±6·0	400·9±7·0	−1·1±2·0	413·6±10·2	405·1±9·6	−1·7±3·1
NZ-105 (10 mg kg^{-1})	SBP	182·7±2·6	140·0±8·1**	−23·4±4·3	205·4±2·6	154·7±5·7**	−24·8±2·2	232·0±2·8	152·3±6·6**	−34·3±2·9
	HR	422·6±6·5	506·0±12·9**	19·7±2·5	384·9±9·5	471·4±11·1**	22·9±4·0	396·0±11·8	472·9±6·5**	20·3±4·9
Propranolol (20 mg kg^{-1})	SBP	183·8±3·2	179·7±3·4	−2·1±2·8	218·0±3·9	220·8±3·1	1·4±1·5	239·8±2·7	244·3±3·8	1·9±1·8
	HR	429·5±5·8	392·2±5·2**	−8·6±2·0	408·8±6·0	360·2±5·7**	−11·8±1·6	418·2±5·0	373·8±3·7**	−10·6±1·2
NZ-105+ propranolol	SBP	183·6±1·8	139·3±4·5**	−24·0±2·9	213·3±1·8	155·7±6·3**	−27·0±2·7	236·0±1·2	144·3±4·8**	−38·9±1·9
	HR	439·6±4·2	412·9±6·0*	−6·0±1·8	373·0±6·4	359·6±9·3	−3·6±2·0	375·1±8·4	371·0±6·1	−0·8±2·8

The values of the SBP and HR are those measured before (pre) and 3 h after (post) the daily administration. Each value represents the mean ± s.e. from 6 to 7 animals. *$P<0·05$, **$P<0·01$ compared with the pre-dose value.

Table 2. Plasma renin activity and plasma aldosterone concentration in SHRs treated with NZ-105, propranolol and both drugs after the administration for 12 weeks.

Drug (dose)	Renin activity (ng mL^{-1} h^{-1})	Aldosterone (pg mL^{-1})
Control	52·1±4·5	736±84
NZ-105 (10 mg kg^{-1})	54·2±6·2	946±226
Propranolol (20 mg kg^{-1})	36·1±4·3	309±49*
NZ-105+propranolol	49·3±6·8	405±63

Each value represents the mean ± s.e. from 6 to 7 animals. *$P<0·05$ compared with the corresponding control values.

administration (Table 1). On the other hand, the tachycardia induced by NZ-105 was completely suppressed by the combined treatment with propranolol, the same as in the single treatment (Fig. 1). The heart rate in the propranolol group was significantly reduced.

The effects on plasma renin activity and plasma aldosterone

The plasma renin activity in NZ-105 and combination groups was almost equal to that in the control group after 12 weeks of administration, while propranolol reduced the activity (Table 2). On the other hand, plasma aldosterone in NZ-105-treated animals did not differ from that in the control group but that in propranolol and combination groups was reduced significantly ($P<0·05$) or tended to decrease, respectively.

Organ weights

The body weights of the SHRs after fasting for 12 h were 324±7·6 g in the control group, 325±9·2 g in the NZ-105 group, 334±4·8 g in the propranolol group and 332±8·2 g in the combination group. These values were not significantly different from each other. The weights of heart, liver and both kidneys after 12 weeks of administration of each drug are summarized in Table 3. In the combination group the heart weight normalized to body weight was significantly ($P<0·05$) less than in the control group. The liver weights in the four groups did not differ significantly. The weights of both kidneys per body weight were significantly ($P<0·01$) reduced in the combination group.

Discussion

NZ-105, a newly synthesized 1,4-dihydropyridine derivative, showed a slow-onset and long-lasting hypotensive effect accompanied by tachycardia in SHRs after a single oral administration (Masuda et al 1990). These observations were confirmed in the present study. As shown in Fig. 1, when combined with propranolol, the hypotensive effect of NZ-105 was not affected (both potency and duration were identical), but tachycardia accompanied by the decrease in blood pressure was prevented. These results were in agreement with those with other calcium antagonists (Warltier et al 1984), and indicate that the tachycardia is evoked via the baroreceptor reflex caused by a decrease in blood pressure. One of the common adverse effects of 1,4-dihydropyridines is palpitation (Doyle 1985); hence the combination of NZ-105 with propranolol may be considered to reduce this side effect.

Long-term administration of drugs is usually necessary in the therapy of hypertension. During therapy, there are two important views; one is that the hypotensive action induced by drugs can be seen during the administration period, and the other is that the increase of systolic blood pressure with age can be suppressed (the antihypertensive effect). As shown in Table 1, the hypotensive action of NZ-105 was reproducible after repeated dosing for 12 weeks, which has been

Table 3. Organ weights of SHRs treated with NZ-105, propranolol and both drugs after administration for 12 weeks.

Drug (dose)	Heart		Liver		Kidney	
	(g)	(mg/100 g)	(g)	(mg/100 g)	(g)	(mg/100 g)
Control	1·34±0·03	415±7·6	9·8±0·3	3024±32·5	2·23±0·07	688±12·7
NZ-105 (10 mg kg^{-1})	1·28±0·03	394±4·8	9·7±0·4	2971±47·1	2·20±0·09	677±8·8
Propranolol (20 mg kg^{-1})	1·32±0·02	396±2·9	10·3±0·2	3072±45·8	2·28±0·04	683±2·4
NZ-105+propranolol	1·27±0·02	383±5·7*	9·7±0·3	2916±33·4	2·07±0·05	625±8·5**

Each value represents the mean ± s.e. from 6 to 7 animals. *$P<0·05$, **$P<0·01$ compared with the corresponding control values.

reported when administered for 4 weeks. In the combination group, propranolol prevented the tachycardia without affecting the hypotensive response to NZ-105 and the effect was observed during 12 weeks.

The increase of predosing systolic blood pressure in the propranolol group did not change in comparison with that in the control group, whereas that in the NZ-105 group was suppressed by repeated administration for 12 weeks. This is in agreement with earlier reports using long-term treatment of other calcium antagonists, amlodipine (Nayler 1988) and nilvadipine (Ohtsuka et al 1989). Furthermore, heart rate in the NZ-105 group was lower than that in the control group. The results were the same in previous experiments in which NZ-105 was given to SHRs for 4 weeks (Masuda et al 1990). It is considered that the reduction in heart rate after repeated administration of NZ-105 may be due to a resetting of the baroreceptor reflex. However, since it has been reported that the basal heart rate was not affected by repeated treatment with 1,4-dihydropyridines in either SHRs (Nayler 1988; Ohtsuka et al 1989) or man (Wever 1988), further detailed investigation is needed. In the combination group, although heart rate tended to be lower than that in the control group, the increase of systolic blood pressure was suppressed only at the second week. As described above, however, the combination with propranolol completely suppressed the tachycardia induced by NZ-105.

The development of hypertension has been reported to be accompanied by cardiac hypertrophy due to left ventricular adaptations to the increased pressure load; the left ventricular weight increased above that expected in normal body growth in SHRs (Pfeffer & Frohlich 1973) or man (Laufer et al 1989). Recently, it has been recognized that the hypertrophy of the heart plays a dominant role as a risk factor in cardiovascular diseases, such as acute myocardial infarction and congestive heart failure (Korner et al 1991; Messerli & Ketelhut 1991). Cardiac hypertrophy is suppressed by long-term treatment of β-adrenoceptor blockers (Ieki et al 1989) or other calcium antagonists (Nayler 1988; Ohtsuka et al 1989). Our results indicate that NZ-105 used in combination with propranolol may suppress cardiac hypertrophy more effectively.

Moreover, kidney weights have also been reported to be increased in SHRs compared with normotensive Wistar rats (Okamoto 1982; Yamori et al 1982). Our study showed that the kidney weights relative to body weights were significantly smaller in the combination group than in the control group. Thus it may be possible that the combination could confer more beneficial effects.

Calcium antagonists increase the plasma renin activity by activating the renal sympathetic nervous system or by increasing circulating catecholamines, both of which are caused by their hypotensive action. After NZ-105 for 12 weeks, the plasma renin activity was about the same as in the control group, suggesting that it had already recovered to the basal level. In the propranolol group the renin activity tended to decrease, which was in agreement with the results shown in a report using higher doses of propranolol (100 mg kg^{-1}) (Kubota et al 1985). On the other hand, there are many reports showing that the plasma aldosterone was not affected by either acute or chronic treatment with calcium antagonists (Fraser et al 1979; Luft et al 1985). Similarly, our experiments showed that plasma aldosterone in the NZ-105 group was not modified compared with that in control group. The plasma aldosterone in the propranolol group was parallel to the plasma renin activity, but the plasma aldosterone in the combination group tended to be lower than that in control group. The reason for this is unclear at present.

In conclusion, long-term administration of NZ-105 in SHR showed antihypertensive effect with lowering heart rate. In combination with propranolol, NZ-105 produced favourable effects such as a decrease in heart and kidney weights per body weight and the suppression of tachycardia by NZ-105. Therefore, although it is not warranted to extrapolate the findings in rats to man, NZ-105 may be a useful antihypertensive drug both in monotherapy as a first-choice drug in the stepped-care approach, and in combination treatment with β-adrenoceptor blockers.

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