CHRONIC EFFECTS OF AROTINOLOL (S-596) IN SPONTANEOUSLY HYPERTENSIVE RATS*

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Arotinolol (S-596, ARL) is a β -adrenoceptor blocking drug with weak α -adrenoceptor blocking activity, and may be classified into the fourth generation. Antihypertensive effects of ARL were studied for 12 weeks in spontaneously hypertensive (SHR) rats. Propranolol (PPL) was used as the reference drug. ARL (20 and 100 mg/kg per day, *p.o.*) and PPL (100 mg/kg per day, *p.o.*) treatments significantly decreased heart rate, within a week after the drug treatments had started and thereafter. Tail blood pressure (BP), determined by prewarming the rat at 50 °C for 3 min, was slightly higher in the two ARL treated groups than in the control. Tail BP was slightly lower in the PPL treated group than in the control. Mean BP determined directly at the 12th week was lower in the two ARL and PPL groups than in the control by more than 20 mmHg. Both ARL (100 mg/kg per day) and PPL (100 mg/kg per day) treatments significantly reduced incidences of the vascular lesions, and also prevented the decrease of kidney weights usually associated with mild vascular lesions. Furthermore, these treatments showed a tendency to decrease plasma renin (PRC) and aldosterone (PAC) concentrations determined 20 h after the last administration. As mean BP must be more reliable than tail BP, it was concluded that ARL (20 and 100 mg/kg per day) showed almost the same chronic antihypertensive activity in SHR rats as PPL (100 mg/kg per day). Preventive effects of ARL on development of vascular lesions also supported the above view.

Keywords—arotinolol; propranolol; blood pressure; heart rate; organ weight; vascular lesion; plasma renin concentration; plasma aldosterone concentration; spontaneously hypertensive rat

INTRODUCTION

Arotinolol (S-596, ARL) is a β -adrenoceptor blocking drug with weak membrane stabilizing and α -adrenoceptor blocking activities and without partial agonist action (PAA).¹⁻⁴ The drug may be classified into the fourth generation,⁵ defined as those with useful clinical features such as direct vasodilatation, or α -adrenoceptor and calcium-entry blockades. ARL is about 1/18 and 5 times more potent in α and β blockades than phenoxybenzamine and propranolol (PPL), respectively.^{2,4}

Development of deoxycorticosterone-salt

hypertension in rats was not attenuated with ARL (20, 50 and 100 mg/kg per day, *p.o.*) within 2 weeks.⁴ Blood pressure (BP) was determined 18 h after the drug administration through a cannula inserted into the abdominal aorta without anesthesia.⁴ However, acute depressor effect was seen 4 to 8 h after oral administration (50 and 100 mg/kg, *p.o.*) on the 10th day of the above experiment.⁴ In spontaneously hypertensive (SHR) rats of 21 to 24-week-old, ARL (10, 20 and 50 mg/kg per day, *p.o.*) decreased BP within 2 d.⁴ The effect lasted for 14 d.⁴ Acute depressor effect was also seen 4, 8 or 24 h after the administration (10,

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20 and 50 mg/kg, *p.o.*) on the first and the 10th day.⁴⁾

Thus, detailed experiment in SHR for longer duration of administration is needed to elucidate antihypertensive effect of ARL, with special reference to superiority of coexistence of α - and β -blocking activity. SHR is known as a good animal model to detect antihypertensive effects of β -blocking drugs.

In the present study, ARL was orally administered to SHR for 12 weeks in doses of 20 and 100 mg/kg per day. PPL was used as the reference drug. Effects on plasma renin (PRC) and aldosterone (PAC) concentrations were also determined.

MATERIALS AND METHODS

Experimental Design — A total of 4 experimental groups, with 9–11 rats in each, were made with combinations of SHR rats and PPL or two doses of ARL. Scheffé's *S* and χ^2 -tests were used for statistical analyses.

Hypertensive Rats — Ten-week-old male SHR rats of F40 from the colony of the Department of Pharmacology, Jichi Medical School, weighing 180–250 g, were used. The drug treatments were started at 11 weeks of age after the control observation for one week.

Determination of Blood Pressure and Heart Rate — Tail blood pressure (BP) and heart rate (HR) were determined by a rat tail manometer-tachometer system (Natsume KN-210). Tail BP and HR were determined without anesthesia after prewarming the rats at 50 °C for 3 min. Tail BP obtained by this apparatus has been shown to be identical with maximum BP (unpublished data). Tail BP and HR were determined once a week during the week before and for 11 weeks after the drug treatments had started. At the end of the experiment, during the 12th week, mean BP was determined directly without anesthesia or restraint through a cannula inserted into the abdominal aorta. The interval between the drug administration and the BP determination was about 20 h.

Drug Treatments — Arotinolol hydrochloride

(Sumitomo Chemical) and propranolol hydrochloride (ICI-Pharma) were administered orally by a gastric tube once a day for 12 weeks. They were suspended in 0.5% methyl cellulose 400 cP (Wako Pure Chemical) solution at a volume of 5 ml/kg body weight. The doses in terms of free bases were 20 or 100 mg/kg for ARL, and 100 mg/kg for PPL respectively. Methyl cellulose solution (5 ml/kg) was given to the control group.

Postmortem Examination — Following BP determination through the aortic cannula, a blood sample of 0.8 ml was obtained for PRC and PAC determinations. Then the rat was sacrificed with ether, and inspected macroscopically. Weights of the heart, kidney, adrenal, liver, spleen and lung were determined. Tissues were fixed with 10% formalin, and embedded in paraffin. Hematoxylin-eosin or elastica-Masson stain was performed on the 4 μ m sections. The vascular lesions in the kidney, heart or mesenteric arteries were examined microscopically. Abnormality in the adrenal, liver, spleen and lung was also examined.

Determination of Plasma Renin and Aldosterone Concentrations — PRC and PAC were determined in the blood samples obtained at postmortem examination by the modified method of Carvalho *et al.*⁶⁾ and the direct radioimmunoassay,⁷⁾ respectively.

RESULTS

Body Weight and Heart Rate

The body weight (BW) of the control and two ARL treated groups increased at approximately an equal rate. The increase of BW was slightly slower in the PPL treated group.

The control groups given the solvent showed fairly constant HR values during the experiments. ARL (20 and 100 mg/kg per day, *p.o.*) and PPL (100 mg/kg per day, *p.o.*) treatments significantly decreased HR, compared to the control, within a week after the drug treatments had started and for 11 weeks thereafter (Fig. 1).

Blood Pressure (Fig. 2)

In the group given the solvent, tail BP continued to increase for 11 weeks. Tail BP values were slightly larger in the two ARL treated

groups than in the control for 11 weeks. They were slightly smaller in the PPL treated group than in the control. However, those differences were not statistically significant for entire period. Mean BP values, determined directly at the 12th week, were smaller in the three groups treated with ARL or PPL than in the control. The differences between the ARL or PPL groups and

the control were more than 20 mmHg. However, neither value was statistically significant.

Postmortem Examination

ARL and PPL treatments significantly affected the organ weights (Table I). Kidney weights were larger in the groups treated with the higher dose of ARL or PPL than in the control. Liver weights were larger in the two groups treated with ARL

FIG. 1. Effects of Arotinolol on HR in SHR Rats
Vertical bars are S.E. of the mean. Initial No. of rats in each group is shown in parentheses following explanation symbols.

--○-- solvent 5 ml/kg, p.o. (11), —●— propranolol 100 mg/kg, p.o. (11), —▲— arotinolol 20 mg/kg, p.o. (10), —■— arotinolol 100 mg/kg, p.o. (9).

FIG. 2. Effects of Arotinolol on BP in SHR Rats
Final No. of rats in each group is shown in parentheses at the last week of the experiments. Other details are the same as in Fig. 1.

--○-- solvent 5 ml/kg, p.o. (11), —●— propranolol 100 mg/kg, p.o. (11), —▲— arotinolol 20 mg/kg, p.o. (10), —■— arotinolol 100 mg/kg, p.o. (9).

TABLE I. Effects of Arotinolol on Organ Weights (g/100g Body Weight) in SHR Rats

Treatment	No. of rats	Heart	Kidney	Adrenal	Liver	Spleen	Lung
Solvent	11 ^{a)}	0.394 ^{b)} ±0.013	0.646 ±0.010	0.017 ±0.001	3.142 ±0.047	0.247 ±0.010	0.710 ±0.028
Propranolol (100 mg/kg)	5	0.394 ±0.012	0.745** ±0.018	0.021* ±0.001	3.346 ±0.057	0.274 ±0.018	0.776 ±0.022
Arotinolol (20 mg/kg)	10	0.364 ±0.005	0.677 ±0.010	0.017 ±0.001	3.514** ±0.052	0.258 ±0.008	0.736 ±0.045
Arotinolol (100 mg/kg)	4	0.377 ±0.007	0.726** ±0.017	0.017 ±0.001	3.680** ±0.127	0.325* ±0.044	0.782 ±0.040

a) The rats died before the termination of the experiment were not included.

b) Figures are mean ± SE.

Statistically significant differences compared to the solvent control group (* $p < 0.05$, ** $p < 0.01$).

than in the control. Adrenal weights were larger in the group treated with PPL than in the control. Spleen weights were larger in the group treated with the higher dose of ARL than in the control. There were no statistically significant differences in heart or lung weights among the four groups.

By macroscopic observation, polyarteritis nodosa in the splanchnic region was seen in each one out of 10 and 9 rats of the groups treated with 20 and 100 mg/kg ARL, respectively. Nephrosclerosis was not observed in either of the four groups. However, vascular lesions were seen microscopically, in 6 out of 11 rats of the control and 2 out of 10 rats of the group treated with 20 mg/kg ARL. This explains the decrease in kidney weights in these groups. PPL and ARL treatments in a dose of 100 mg/kg prevented vascular lesions and loss of kidney weights. These vascular

lesions in the splanchnic region and kidney are referred as the vascular disease usually observed in hypertensive rats.

No abnormality was seen microscopically in the liver of the two groups treated with ARL, nor in the adrenal of the group treated with PPL, compared with those of the control group. Increase of splenic weight in the group treated with 100 mg/kg ARL may be sequelae of multiple granulomatous lesion.

Plasma Renin and Aldosterone Concentrations (Figs. 3 and 4)

Both PRC and PAC values were smaller in the groups treated with the higher dose of ARL and PPL than in the control. However, there were no statistically significant differences. There were no significant correlation between mean BP and PRC ($r^2=0.048$) or PAC ($r^2=0.150$).

DISCUSSION

In the BP determination, discrepancy between BP values determined directly and indirectly became apparent after administration of β -adrenoceptor blocking drugs. Especially, tail BP were slightly larger in the two groups treated with

FIG. 3. Effects of Arotinolol on Plasma Renin Concentration in SHR Rats

Vertical bars are S.E. of the mean. No. of rats are shown at the bases of columns. PPL: propranolol. ARL: arotinolol.

FIG. 4. Effects of Arotinolol on Plasma Aldosterone Concentration in SHR Rats

Details are the same as in Fig. 3.

ARL than in the control at the 11th week, whereas mean BP values were smaller in them than in the control by more than 20 mmHg at the 12th week. We have shown that prewarming at 50 °C for 3 min increases tail BP and mean BP simultaneously in the rats treated with β -adrenoceptor blocking drugs (unpublished data). Therefore, this discrepancy should be due to the prewarming condition in indirect BP determination. We have also shown that there was better prewarming condition which did not produce such a discrepancy in the rats treated with β -adrenoceptor blocking drugs (unpublished data).

Therefore, in the present study, mean BP determined directly without anesthesia or restraint is more reliable than tail BP determined indirectly after prewarming. Thus, it was concluded that ARL (20 and 100 mg/kg per day, *p.o.*) treatments showed almost the same chronic antihypertensive effects in SHR rats as PPL (100 mg/kg per day, *p.o.*) treatments did. However, superiority of coexistence of α - and β -blocking activities was not demonstrated in the present experiment.

Both ARL (100 mg/kg per day) and PPL (100 mg/kg per day) treatments significantly reduced incidences of the vascular lesions in the kidney and mesenteric artery in SHR rats. However, these treatments increased incidences of death during the experiments (figures are shown in Fig. 2). Postmortem examination revealed higher incidences of infections such as lung abscess, bronchitis and pyelonephritis, which may be resulted from toxic effects of PPL and ARL in these groups.

Kidney weight is known to be rather increased in association with severe vascular lesions, whereas it is decreased in association with mild lesions. Therefore, it is appropriate that kidney weights decreased in the control and 20 mg/kg ARL groups because of vascular lesions.

Previous reports indicated that PPL (100 mg/kg per day) treatments for 11 weeks decreased heart weights in SHR rats.^{8,9)} In the present study, PPL treatment, as well as ARL treatment, did not decrease heart weights signifi-

cantly. The reason for this discrepancy is not known.

ARL and PPL treatments did not significantly decreased PRC nor PAC in SHR rats, when the blood samples were obtained in 20 h after the last administration. In the previous reports, it was shown that PPL (100 mg/kg) treatment significantly decreased PRC in SHR rats, when the blood samples were obtained in 3 or 6–9 h after the last administration.^{9,10)} Therefore, it was likely that β -adrenoceptor blocking activity of ARL and PPL were reduced after 20 h, and PRC and PAC were recovered. No significant correlation existed between mean BP and PRC or PAC values in the present study. This might suggest that participation of the renin-angiotensin-aldosterone system in the pathogenesis of hypertension and antihypertensive effect of ARL or PPL in SHR rats was limited, as shown elsewhere.^{6,11)}

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