ACUTE AND SUBCHRONIC EFFECTS OF BOMETOLOL ON BLOOD PRESSURE IN HYPERTENSIVE RATS*

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Bometolol is a cardiospecific β -adrenergic blocking drug. The effect on blood pressure was determined in hypertensive rats in acute and subchronic experiments. In acute experiment bometolol, 10–30 mg/kg, *p.o.*, was given to spontaneously hypertensive rats; deoxycorticosterone and salt hypertensive rats; and two kidney, one clip hypertensive rats. Blood pressure was determined continuously for 8 h and after 24 h in unanesthetized condition. The blood pressure lowered by bometolol was dose-dependent in three types of hypertension. In subchronic experiment, bometolol at a dose of 10–30 mg/kg or 100–300 mg/kg, *p.o.*, were given for 5 weeks to deoxycorticosterone and salt hypertensive rats; and two kidney, one clip hypertensive rats. Bometolol did not show antihypertensive effect in either type of hypertension, although bometolol decreased heart rate dose-dependently. Bometolol treatment at the above doses decreased plasma renin activity, heart and kidney weights, and incidence of vascular lesion in either hypertensive rat. Toxic symptom was seen 3 days after bometolol treatment of 300 mg/kg in deoxycorticosterone and salt hypertensive rats.

Keywords—bometolol; blood pressure; heart rate; plasma renin activity; spontaneously hypertensive rats; deoxycorticosterone and salt hypertensive rats; two kidney, one clip hypertensive rats

INTRODUCTION

Bometolol (BML), 8-acetonyloxy-5-[3-{2-(3,4-dimethoxyphenyl) ethylamino}-2-hydroxypropoxy]-3,4-dihydrocarbostyryl (OPC-1427) is a cardiospecific β -adrenergic blocking drug.¹⁾ Intravenous injection of BML in normotensive dogs, cats, and rats, or two kidney, one clip hypertensive rats acutely decreased blood pressure.^{1,2)} BML, 10–30 mg/kg, given orally into one kidney, one figure-8, hypertensive dogs also acutely decreased blood pressure.³⁾ Hypotensive action was also observed during 24 h after intraperitoneal injection of BML, 0.5–10 mg/kg, into normotensive rats of Wistar-Kyoto strain (WKY), spontaneously hypertensive rats (SHR), and deoxycorticosterone and salt hypertensive rats (DOC).⁴⁾ The results may be relatively

unreliable, because blood pressure was determined by the tail cuff method and prewarming the rats every one hour for 6 hours.

The chronic treatment with β -adrenergic blocking drugs are known to lower blood pressure only in the SHR rats among various types of hypertensive rats.^{5–9)} In the present study we re-examined the acute depressor effect for 24 h in normal, SHR, DOC, and two kidney, one clip (CLIP) hypertensive rats without anesthesia or restraint. We also determined antihypertensive effect of BML for 5 weeks in DOC and CLIP hypertensive rats, in which β -adrenergic blocking drugs are not effective.^{6–9)}

MATERIALS AND METHODS

Experimental Design — Present study consists

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of two major parts, acute and subchronic experiments. In the acute study, a total of 12 experimental groups, 6–8 rats each, made by different combination of hypertensive rats and doses of BML were studied. Six groups, 9–12 rats each, of different combination of DOC and CLIP rats and doses of BML were used in the subchronic experi-

ments. Scheffé's S and χ^2 -tests were used for statistical analyses.

Hypertensive Rats — In the acute experiments, normal HOS®: Donryu strain (DON), and SHR, DOC, and CLIP hypertensive rats were used. The subchronic studies were performed in DOC and CLIP hypertensions. All rats were female. DON

FIG. 1. *Acute Effects of Bometolol on Mean Blood Pressure in Normotensive DON Rats*

Vertical bars are SE of the mean. No. of rats are in parentheses following explanation symbols.

FIG. 2. *Acute Effects of Bometolol on Mean Blood Pressure in SHR Rats*

Details are the same as in Fig. 1.

FIG. 3. *Acute Effects of Bometolol on Mean Blood Pressure in DOC Hypertensive Rats*

Details are the same as in Fig. 1.

FIG. 4. *Acute Effects of Bometolol on Mean Blood Pressure in CLIP Hypertensive Rats*

Details are the same as in Fig. 1.

strain were 14–15 weeks of age, weighing 250–270 g. SHR rats were 17–18 weeks of age, F34 from the colony of the Department of Pharmacology, Jichi Medical School, weighing 180–200 g. DOC and CLIP hypertensions were made in DON strain rats, 8–9 weeks of age, weighing 180–210 g, as reported previously.⁹⁾ They were used at 7–9 and 6 weeks after the surgery or initiation of DOC treatment in the acute and subchronic experiments, respectively.

Determination of Blood Pressure and Heart Rate — In the acute experiment, the mean blood pressure (BP) was determined directly without anesthesia or restraint through a cannula inserted into the abdominal aorta a day before.¹⁰⁾ After the control determination of BP for 15 min before drug administration, BP was recorded continuously for 8 h and the value at 24 h was taken separately. BP values were read every 0.5 h for the first 3 h and every 1.0 h for 4–8 h after the treatment. In the subchronic experiments, tail BP and heart rate (HR) were determined as reported previously⁹⁾ once a week immediately before and for 4 weeks after the drug treatments started. The intervals between the drug administration and the determinations were 3–6 h, and randomized in each rat. At the end of the drug treatments, during the 5th week, the mean BP was determined directly as described above.

Drug Treatments — Bometolol hydrochloride (Otsuka Pharmaceutical) was dissolved in H₂O and 0.01% Tween 80 solution at a volume of 5 ml/kg body weight for the acute and subchronic studies, respectively. The doses were in terms of the free base. The solution was administered orally by a gastric tube. In the acute experiment, H₂O (5 ml/kg) was given to the control groups. In the subchronic experiment, the drug was administered once a day for 5 weeks, 5 days per week, and Tween 80 solution (5 ml/kg) was given to the control groups.

Determination of Plasma Renin Activity and Postmortem Examination — In the subchronic studies, a blood sample of 0.5 ml was obtained following BP determination through the aortic cannula, and used for determination of plasma

renin activity (PRA). PRA was determined by the method of Carvalho *et al.*¹⁰⁾ The detecting limit of this method is 1.7 ng/ml per h. The rat was then sacrificed with ether, and inspected macroscopically. The heart and kidney were weighed before fixation.

RESULTS

A. Acute Effects of BML in Hypertensive Rats (Fig. 1–4)

In normotensive DON rats, BP of the control group treated with H₂O was unchanged throughout the observation period of 8 h and after 24 h. BML treatment, 30 or 100 mg/kg, *p.o.*, decreased

FIG. 5. Subchronic Effects of Bometolol given orally for 4 Weeks on Heart Rate in DOC Hypertensive Rats

Initial No. of rats in each group is in parentheses following explanation symbols. Final No. is shown in parentheses at the 4th week. Dose of each drug was increased stepwise as shown in a staircase belt. Other details are the same as in Fig. 1.

BP slightly, but the effect was not dose-dependent. Statistically significant differences ($p < 0.05$) were obtained against the control group at 0.5–8 h after the treatment with 30 mg/kg, and at 1–2.5 h after 100 mg/kg, respectively. BP returned to the initial values after 24 h.

SHR rats showed a slight decrease in BP after H₂O treatment (control group). BML, 30 and 100 mg/kg, *p.o.*, produced a dose-dependent decrease in BP. In both treated groups, BP continued to decrease for the first 1–2 h, and the effect lasted for 6–7 h. BP returned nearly to the control value at 24 h after the treatment. The differences from control values (H₂O group), at 0.5–8 h after the treatment with either dose, were statistically significant ($p < 0.05$).

DOC hypertensive rats also showed a slight decrease in BP at 5–8 h after H₂O treatment.

Relatively short fall for 4 h after the treatment was seen in the rats treated with 30 mg/kg of BML. With 100 mg/kg, two phases of BP decrease in about 20 mmHg from the control were observed. However, neither value after the treatment of 30 or 100 mg/kg was statistically significant against the control value.

CLIP hypertensive rats treated with H₂O or 30 mg/kg of BML showed fairly constant BP values for 24 h. BP decreased after administration of 100 mg/kg BML, and the decrease continued for 24 h. Differences at 1.5–3, 5, 6, and 8 h after the treatment were statistically significant ($p < 0.05$) against the control.

B. Subchronic Effects of BML in Hypertensive Rats

BML treatments were carried out in each three groups of DOC and CLIP hypertensive rats. Doses of BML were 10 or 100 mg/kg, *p.o.*, for the

FIG. 6. Subchronic Effects of Bometolol given orally for 4 Weeks on Heart Rate in CLIP Hypertensive Rats

Details are the same as in Fig. 5.

FIG. 7. Subchronic Effects of Bometolol Given orally for 5 Weeks on Blood Pressure in DOC Hypertensive Rats

Details are the same as in Fig. 5.

first 2 weeks and 30 or 300 mg/kg, *p.o.*, thereafter. BML treatments at doses of 10–30 and 100–300 mg/kg were designated as low and high doses, respectively. In DOC rats, the treatment with 300 mg/kg of BML was withdrawn for 3 days from the 3rd to 5th day in the 4th week because toxic symptom had appeared in 4 out of 8 rats. Rats were judged to be intoxicated when they crouched with hyperpnea with decreased spontaneous movements. The groups given the solvent served as the controls.

Body Weight and Heart Rate (Fig. 5, 6) — The body weight of each three groups of differently treated DOC and CLIP rats increased gradually at an almost equal rate, except at the 4th week in DOC rats with high doses, which showed toxic symptom. The control groups given the solvent showed fairly constant HR values during the experiments. In DOC rats, BML treatments showed dose-dependent decreases in HR, except at the 4th week of treatment with 300 mg/kg of BML during which the treatment had been withdrawn for 3 days. In CLIP rats, BML treatments resulted in dose-dependent decreases in HR. All of differences in HR after BML treatments in DOC and CLIP rats were statistically significant ($p < 0.005$) against the controls.

Blood Pressure (Fig. 7, 8) — In DOC hypertension, BP of the solvent control group was usually higher than 190 mmHg during the 5 weeks of experiment. The low doses of BML did not change the BP values. A significant difference ($p < 0.025$) was only seen at the 3rd week with the highest dose (300 mg/kg) of BML. The BP value returned to almost the initial value at the 4th week during which the treatment was withdrawn for 3 days. Effect of BML given 3 days before the determination must be minimal as judged by HR values determined at the same time, which recovered nearly to the initial level. Mean BP determined during the 5th week after 300 mg/kg of BML for 3 days were about 30 mmHg lower than the control but the differences was not statistically significant.

In the CLIP hypertension, BP of the control group was 210 mmHg in average for 4 weeks.

Mean BP values at the 5th week was even higher than BP determined indirectly at the tail at the 4th week, but the difference was not statistically significant. The reason for this rise was not known. The treatment with low doses of BML did not change tail BP throughout 4 weeks. The mean BP at the 5th week was significantly lower than the control ($p < 0.01$) because of a sudden rise of BP in the control group. BP of the rats treated with high doses of BML did not differ significantly from the control. The reason as to why relatively higher value was seen at the 3rd week with the highest dose of BML (300 mg/kg) is unknown. Mean BP determined at the 5th week was lower than the control ($p < 0.05$).

Plasma Renin Activity and Findings at Postmortem Examination

PRA was decreased by BML treatment in

FIG. 8. Subchronic Effects of Bometolol given orally for 5 Weeks on Blood Pressure in CLIP Hypertensive Rats

Details are the same as in Fig. 5.

DOC and CLIP hypertensions, although the differences were not statistically significant (Table I).

Vascular lesions like periarteritis nodosa were seen in the mesenteric area of DOC and CLIP rats. BML treatments showed a tendency to decrease incidences of the vascular disease in either type of hypertension (Table II). BML treatment with higher doses decreased heart and kidney weights in DOC and CLIP rats (Data was not shown).

DISCUSSION

In the acute experiments, decrease of BP of animals treatment with BML 30–100 mg/kg, *p.o.*, was dose-dependent in SHR, DOC, and CLIP hypertensive rats under unanesthetized

condition. The BP decrease continued for 8 h in SHR and DOC, and 24 h in CLIP treated with 100 mg/kg of BML. Prolonged BP effect of BML was only seen in CLIP rats after the treatment with 100 mg/kg. Judging from the general appearances on or following the day, this effect was not due to intoxication or weakness of the rats. The same treatment lowered BP only slightly in normotensive DON rats, but the effect was not dose-dependent. The results are basically in accord with the data of previous reports on BML,¹⁻⁴⁾ although details of the experimental conditions are different from each other.

In the subchronic experiments, treatment with BML 10–300 mg/kg per day, *p.o.*, for 5 weeks did not lower BP significantly in either DOC or

TABLE I. *Effects of Bometolol on Plasma Renin Activity*

Hypertension	Treatment	PRA (ng/ml per h)	<i>p</i>
DOC	SOL	3.6 ± 1.9 (8) ^{a)}	—
	BML-L	0 (9)	NS ^{b)}
	BML-H	0 (7)	NS
CLIP	SOL	229 ± 107 (7)	—
	BML-L	93 ± 28 (12)	NS
	BML-H	98 ± 28 (8)	NS

a) Mean ± SE. No. of rats are in parentheses.

b) Statistically not significant compared to SOL. SOL: Control given the solvent, BML-L: Treatment with low doses of bometolol (10–30 mg/kg, *p.o.*), and BML-H: Treatment with high doses of bometolol (100–300 mg/kg, *p.o.*).

TABLE II. *Effects of Bometolol on Incidence of Vascular Disease and Related Death*

Hypertension	Treatment ^{a)}	Vascular disease	Death ^{b)}
DOC	SOL	4/9	0/9
	BML-L	2/10	1/10
	BML-H	0/10	0/10
CLIP	SOL	9/12	3/12
	BML-L	4/12	0/12
	BML-H	3/10	1/10

a) See Table I for detail.

b) Death refers to that related to the vascular disease.

CLIP hypertensive rats. The only exception is that DOC rats, intoxicated with 300 mg/kg of BML showed significant decreases in BP. In CLIP rats, mean BP values determined directly at the 5th week were significantly lower in groups treated with 30 or 300 mg/kg of BML than the untreated control. This was caused by a sudden rise in the BP of control rats.

Considering the above factors, we concluded that subchronic treatment with BML up to 300 mg/kg per day for 5 weeks did not lower BP. The results are basically in accord with the previous reports on DOC or CLIP hypertensive rats treated with other β -adrenergic blocking drug.⁵⁻⁹ Effects of BML were apparent at least on the heart when BP was determined, because HR decrease was dose-dependent in both DOC and CLIP rats. The reason for the discrepancy between acute and subchronic effects of BML on BP is unknown.

Resistance of these hypertensive rats to antihypertensive effect of β -blocking drugs could be explained by the decreased affinity or number of cardiac adrenergic receptors.^{11,12} Relatively larger doses of the drug required to lower cardiac output might increase total peripheral resistance by blocking vascular β -receptors. The above explanation is possible only when decreased cardiac output and subsequent autoregulation is the main mechanism of antihypertensive effect.

PRA decreased with BML treatments for 5 weeks in DOC and CLIP rats. Although the results are conflicting as to the β -receptor subtypes to release renin from the juxtaglomerular cells,¹³⁻¹⁵ the present results suggest that β_1 -receptors participate in the release of renin.

Decreasing tendency of vascular lesions like periarteritis nodosa in both types of hypertension after BML treatments are interesting. As BP did not change significantly, BML might have some unknown protecting action against development of vascular lesion in hypertensive rats. However, as vascular lesion was examined macroscopically in the present study, further studies are necessary to elucidate this effect of BML.

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