Antihypertensive Effect of Repeatedly Administered YM358, an Angiotensin AT₁-Receptor Antagonist, in Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT—YM358 (2,7-diethyl-5-[[2'-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]-triazole potassium salt), a novel nonpeptide angiotensin AT₁-receptor antagonist, was administered daily for 4 weeks to 24-week-old stroke-prone spontaneously hypertensive rats (SHRSP). Its effects on systolic, mean and diastolic arterial pressure (SAP, MAP and DAP), heart rate and locomotor activity were investigated by using radiotelemetry. A clear diurnal variation in blood pressure, heart rate and locomotor activity was observed in synchrony with the light cycle. YM358 at a daily oral dose of 10 or 30 mg/kg produced a reduction of blood pressure in a dose-dependent manner. Although a mild attenuation of the antihypertensive effect of YM358 was observed during the early stage of therapy, YM358 at 30 mg/kg per day produced a significant and consistent decrease in 24-hr MAP and DAP, and it prevented the further development of hypertension. YM358 did not affect either heart rate or locomotor activity or their diurnal variations. After the discontinuation of therapy with YM358, the blood pressure recovered promptly to the control level while there was no sign of a rebound increase in blood pressure. These results suggest that YM358 may be potentially useful for the treatment of hypertension.

Keywords: YM358, Angiotensin AT₁-receptor antagonist, Antihypertensive effect, Stroke-prone spontaneously hypertensive rats, Radiotelemetry

YM358 (2,7-diethyl-5-[[2'-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole potassium salt) is a novel nonpeptide angiotensin AT₁-receptor antagonist. Its pharmacology has been reported by Fujimori et al. (1–3), Shibasaki et al. (4), Okazaki et al. (5), Tokioka et al. (6, 7) and Kusayama et al. (8). In renal hypertensive (2K1C) rats (RHR) and spontaneously hypertensive rats (SHR), a single oral administration of YM358 at 10 or 30 mg/kg produced a dose-dependent and long-lasting decrease in blood pressure without affecting heart rate (4, 6, 7). Since YM358 is a competitive AT₁ antagonist (1, 5), it has been suggested that the increase in plasma renin concentration (PRC) will lead to an increase in plasma angiotensin II concentration that may attenuate the antihypertensive effect of YM358 during repeated administration, and may thus induce a rebound increase in blood pressure after discontinuation of therapy. Most studies on the long-term effects of angio-

tensin II-receptor antagonists on blood pressure have been performed by the tail-cuff method (9–13). However, little information is available on the effect of angiotensin II-receptor antagonists on 24-hr continuous blood pressure during repeated administration (14).

In the present study, we have investigated the effect of daily administration of YM358 for 4 weeks on the diurnal variation in blood pressure, heart rate and locomotor activity using radiotelemetry in 24-week-old stroke-prone SHR (SHRSP). In addition, measurements were continued for 7 days after the discontinuation of YM358 to determine whether there was a rebound increase in blood pressure.

MATERIALS AND METHODS

YM358 was synthesized and supplied by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). Solutions of YM358 were prepared freshly each day just before use by dissolving it in distilled water. In a previous study, a single

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oral administration of YM358 at 10 and 30 mg/kg to RHR and SHR caused a marked and significant decrease of blood pressure as determined directly via an aortic catheter (4). Thus, we selected oral doses of YM358 at 10 and 30 mg/kg per day in the present study.

Procedures in animals

This study was carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Male 21-week-old SHRSP from the strain of Okamoto et al. (15) were acclimatized to a 12-hr light/dark cycle (lights on at 08:00 hr). They were allowed free access to a rat chow containing 0.29% (W/W) sodium and 0.57% (W/W) potassium (CE-2; Clea Japan, Inc., Tokyo) and to tap water for drinking.

Systolic, mean and diastolic arterial pressure (SAP, MAP and DAP), heart rate and locomotor activity were measured telemetrically with the Dataquest IV system (Data Sciences International, Roseville, MN, USA). The monitoring system consists of an implantable transmitter, receiver panel, consolidation matrix, and an IBM-compatible personal computer with accompanying software (14, 16–18).

At 22 weeks of age, the rats were anesthetized with 50 mg/kg pentobarbital sodium, i.p. The tip of a catheter which refers pressure to a sensor consisting of an implantable transmitter was inserted and secured in the abdominal aorta about 5 mm below the renal artery. The transmitter was sutured to the abdominal wall. The rats were housed in individual polycarbonate cages after surgery. Each cage was placed on a receiver panel connected to the personal computer to allow data storage. With this radiotelemetry system, the rats are completely unrestrained and free to move about in their individual cages. Data relating to hemodynamic parameters and locomotor activity were sampled every 10 min in each rat as a wave form curve for 10 sec. About 10 to 12 days were required after surgery for recovery to the preoperative weight and the re-establishment of a clear diurnal variation in hemodynamic parameters and locomotor activity. Thus, 2 weeks after surgery, the rats were divided into three groups of 6–8 animals each and administered 10 or 30 mg/kg of YM358 or distilled water (2 ml/kg) by gavage once daily for 4 weeks at 19:00 hr. The rats were also observed for 7 days after discontinuation of drug therapy.

Calculations and statistical analyses

The results are presented as means $\pm$ S.E.M. Statistical analyses of the differences between the control and drug-treated groups were assessed by analysis of variance followed by the Bonferroni method (19). A P value of less

than 0.05 was considered to be statistically significant.

RESULTS

YM358 slightly enhanced the growth of SHRSP during and after therapy, but the difference in body weight between YM358-treated rats and the control rats was not statistically significant (data not shown).

A clear diurnal variation in MAP, heart rate and locomotor activity was observed in synchrony with the light cycle before the start of therapy (Fig. 1). MAP was higher during the dark phase than during the light phase and decreased clearly after the lights went on. Heart rate and locomotor activity were markedly higher during the dark phase than during the light phase, decreasing sharply soon after the lights went on and increasing rapidly after the lights went off.

Twenty-four-hour patterns of MAP, heart rate and locomotor activity on day 1 of therapy are shown in Fig. 2. YM358 at doses of 10 and 30 mg/kg administered at 19:00 hr caused a significant decrease in MAP which lasted for about 21 hr after administration ($P < 0.05$ or $P < 0.01$). There were no apparent differences in the degree of decrease in MAP between the doses. YM358 did not affect either heart rate or locomotor activity or their diurnal variations.

Figure 3 shows the effects of YM358 on 24-hr SAP, MAP and DAP during and after therapy. In control rats, 24-hr SAP was maintained at around 240 mmHg during therapy for 28 days, whereas 24-hr MAP and DAP increased gradually by about 5 mmHg from about 205 and 170 mmHg, respectively. YM358 produced a decrease in 24-hr SAP, MAP and DAP in a dose-dependent manner throughout therapy. However, the degree of decrease in 24-hr SAP by YM358 was less marked than those in 24-hr MAP and DAP, probably because 24-hr SAP in control rats was unstable, as seen from its large S.E.M. At 10 mg/kg per day, YM358 produced a significant decrease in 24-hr SAP, MAP and DAP during the early stage of therapy ($P < 0.05$ or $P < 0.01$), while attenuation of the decrease in 24-hr SAP, MAP and DAP was observed during the first week of therapy. At 30 mg/kg per day, YM358 caused a significant decrease in 24-hr SAP, MAP and DAP ($P < 0.05$ or $P < 0.01$). While gradual development of the attenuation of the decrease in 24-hr SAP, MAP and DAP was also observed during the first week of therapy, YM358 at 30 mg/kg per day maintained 24-hr MAP and DAP around 170 and 140 mmHg, respectively, from the second to fourth week of therapy. After discontinuation of therapy, 24-hr SAP, MAP and DAP promptly approached the control levels, but did not exceed the control levels during the observation period of 7 days.

Fig. 1. Diurnal variation in MAP, heart rate (HR) and locomotor activity in 24-week-old SHRSP rats before therapy. The open and solid bars indicate light and dark phases, respectively. Values are means $\pm$ S.E.M. for rats allotted to Control ($\bullet$, $n=6$), YM358: 10 mg/kg ($\blacksquare$, $n=7$) or 30 mg/kg ($\blacktriangle$, $n=8$).

Fig. 2. Effect of YM358 or vehicle on the 24-hr patterns of MAP, heart rate (HR) and locomotor activity in SHRSP on day 1 of therapy. YM358 or vehicle was administered by gavage at 19:00 hr. The open and solid bars indicate light and dark phases, respectively. Values are means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, compared with the control. Control (●, $n = 6$); YM358: 10 mg/kg (■, $n = 7$), 30 mg/kg (▲, $n = 8$).

Fig. 3. Effect of repeated administration of YM358 or vehicle on the 24-hr SAP, MAP and DAP in SHRSP. Values are means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, compared with the control. Control (●, $n=6$); YM358: 10 mg/kg (■, $n=7$), 30 mg/kg (▲, $n=8$).

Fig. 4. Effect of repeated administration of YM358 or vehicle on the 24-hr heart rate (HR) and locomotor activity in SHRSP. Values are means $\pm$ S.E.M. Control (●, $n=6$); YM358: 10 mg/kg (■, $n=7$), 30 mg/kg (▲, $n=8$).

Figure 4 shows the 24-hr patterns of heart rate and locomotor activity during and after therapy. YM358 at both doses did not affect either heart rate or locomotor activity.

DISCUSSION

The results of the present study demonstrate that repeated administration of YM358, a nonpeptide AT_1 -receptor antagonist, produces an antihypertensive effect without affecting the growth or diurnal variation in heart rate and locomotor activity in adult SHRSP. Although a mild attenuation of the antihypertensive effect of YM358 at doses of 10 and 30 mg/kg was observed during the early stage of repeated administration, the decrease in

MAP at 30 mg/kg per day remained significant throughout therapy. After discontinuation of the therapy, blood pressure recovered promptly to the control level, but there was no sign of a rebound increase in blood pressure.

A relationship between the continuous increase in blood pressure and the activated renin-angiotensin system has been suggested in the adult SHRSP, because the SHRSP in the advanced stages of hypertension had an elevated PRC (20, 21). The major mechanism of the antihypertensive effect of YM358 is likely to be the blockade of the vasoconstrictor action of angiotensin II through antagonism at AT_1 receptors in the vascular smooth muscle (1, 5). In the present study, YM358 at 30 mg/kg per day decreased 24-hr MAP to 170 mmHg and maintained this decrease from the second to the fourth week of thera-

py, and thus prevented the continuous increase in MAP. This finding further confirms that angiotensin II is involved in the progressive and sustained increase in MAP which is typically observed in the adult SHRSP (20, 21).

It has been suggested that the increase in PRC and the resultant increase in angiotensin II concentration at the receptor sites in vascular smooth muscle may attenuate the antihypertensive effect of competitive type AT₁-receptor antagonists such as YM358 (1, 5) during repeated administration and may induce a rebound increase in blood pressure after discontinuation of therapy. YM358 (1 to 30 mg/kg) caused a dose-dependent decrease in blood pressure in RHR and SHR after a single oral administration in previous studies (4, 6, 7), while in the present study, YM358 at 10 and 30 mg/kg caused a significant and almost the same degree of decrease in MAP of SHRSP on day 1 of therapy. However, mild attenuation of the antihypertensive effect of YM358 was observed during the early stage of therapy in the present study. The degree of the attenuation was more apparent at 10 mg/kg per day than that at 30 mg/kg per day, with 30 mg/kg per day producing a relatively stable antihypertensive effect between the second and fourth week of therapy. In a previous study, YM358 produced an increase in PRC in rats and renal hypertensive dogs (4) after single oral administration. Further, a compensatory increase in PRC has been reported with other AT₁-receptor antagonists in rats (13, 22, 23) and man (24). One of the possible reasons for the mild attenuation of the antihypertensive effect observed during repeated administration of YM358 can therefore be attributed at least in part to the increase in PRC. Another possibility is that the metabolic clearance of YM358 may be accelerated by induction of drug metabolizing enzymes during repeated administration. However, this possibility is unlikely because the plasma half-life of YM358 in Fischer rats was about 2 hr after a single oral administration, and no changes in the plasma half-life of YM358 were observed during and after repeated administration for 8 days (M. Kayama et al., unpublished data).

Losartan and TCV-116 are reported to be converted after absorption into more active metabolites, namely EXP 3174 and CV-11974, respectively, which cause insurmountable inhibition of angiotensin II-induced vascular constriction (25, 26). This insurmountable inhibition by these active metabolites is thought to contribute to the prolonged antihypertensive effect of these drugs and to be advantageous in preventing a rebound increase in blood pressure which may occur when AT₁-receptor antagonists cause an increase in plasma angiotensin II concentrations (27, 28). In fact, a prolonged antihypertensive action of losartan has been observed after discontinuation of therapy in young SHRSP administered losartan at

a dose of 10 mg/kg per day for 9 weeks (11). A sufficient dose of YM358 prevented the development of hypertension and did not show any sign of a rebound increase in blood pressure after discontinuation of therapy in the present study. A formation of an active metabolite with a competitive type AT₁-receptor antagonistic action was recognized after the oral administration of YM358 in rats. However, after an oral administration of ¹⁴C-YM358, the plasma half-life of the radioactivity was about 2.5 hr, suggesting that the elimination of the metabolites from plasma is rapid as well as that of YM358 (M. Kayama et al., unpublished data). Thus, the active metabolite does not appear to contribute much to prolongation of the hypotensive action of YM358. Furthermore, KT3-671, another competitive type of AT₁-receptor antagonist, also induced no rebound increase in blood pressure in SHRSP (14). These findings suggest that even a competitive type of AT₁-receptor antagonist does not always cause a rebound increase in blood pressure after discontinuation of therapy.

Heart rate and locomotor activity in SHRSP showed a clear diurnal variation in synchrony with the light cycle. Despite a significant reduction of blood pressure, YM358 did not cause reflex tachycardia as reported for other AT₁-receptor antagonists (9, 13, 23).

Losartan has been reported to have no sedative effect in mice (29). YM358 did not affect either locomotor activity or their diurnal variations during and after discontinuation of therapy, suggesting that YM358 has no sedative effects.

In summary, repeated oral administration of YM358, a novel nonpeptide AT₁-receptor antagonist, at 30 mg/kg per day for 4 weeks produced a consistent antihypertensive effect without affecting the diurnal variation in heart rate and locomotor activity in adult SHRSP. Mild attenuation of the antihypertensive effect of YM358 was seen during the early stage of therapy, but there was no sign of a rebound increase in blood pressure after discontinuation of therapy. These results suggest that YM358 has an antihypertensive effect of potential usefulness for the treatment of hypertension.

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