EFFECTS OF YS-980, AN ORALLY ACTIVE CONVERTING ENZYME INHIBITOR, ON BLOOD PRESSURE IN NORMOTENSIVE AND HYPERTENSIVE RATS

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Abstract—The hypotensive effect of YS-980, (4R)-3-[(2S)-3-mercapto-2-methyl propanoyl]-4-thiazolidinecarboxylic acid, was investigated in normotensive and different models of hypertensive rats. Experiments were carried out in both anesthetized rats (1 mg/kg i.v.), and conscious unrestrained rats (10 mg/kg p.o.). YS-980 markedly lowered blood pressure in acute renal hypertensive and two-kidney, one-clip hypertensive rats and moderately lowered the pressure in SHR. Contrary to these hypotensive effects, this agent did not reduce the blood pressure in DOCA hypertensive rats. In normotensive rats, YS-980 had a slight hypotensive action in anesthetized rats but not in the conscious animals. The hypotensive effect of YS-980 is attributed mainly to suppression of the renin-angiotensin system.

The renin-angiotensin system appears to be involved in different physiological processes including the control mechanisms of blood pressure, thirst and sodium excretion. However, the exact role of this system has not been completely clarified. Availability of a specific inhibitor of this system would be useful not only to clarify the physiological and pathological role of the renin-angiotensin system, but also to treat the hypertensive patient with high levels of renin.

Many renin inhibitors, converting enzyme (CE) inhibitors and angiotensin II antagonists have been reported already (1). Among them, an orally active CE inhibitor (captopril) has been studied extensively in hypertensive animals and in hypertensive patients (2, 3).

It is well known that CE (kininase II) converts biologically inactive angiotensin I (AI) to active angiotensin II (AII) and degradates biologically active bradykinin to inactive fragments. Therefore, CE inhibitor inhibits the degradation of bradykinin as well as the conversion of AI to AII. In addition, bradykinin stimulates prostaglandins synthesis (4). Therefore, the antihypertensive action of CE inhibitor may to some extent be attributable to effects of the kallikrein-kinin system and to prostaglandins.

YS-980, (4R)-3-[(2S)-3-mercapto-2-methyl propanoyl]-4-thiazolidinecarboxylic acid, is an orally active CE inhibitor with a potency almost similar to that of captopril, D-3-mercapto-2-methylpropanoyl-L-proline, and inhibits the conversion of AI to AII and degradation of bradykinin both in vitro and in vivo (5-7). It has also been reported that YS-980 increased the renal blood flow, urine flow and urinary excretion of sodium. These renal effects evoked by YS-980 were abolished after the inhibition of kallikrein, as induced by aprotinin (8). In the present
study, the hypotensive effects of YS-980 were assessed in normotensive, acute renal, two-kidney, one-clip, DOCA treated, and spontaneously hypertensive rats.

MATERIALS AND METHODS

Experimental animals

Normotensive rats: Male Wistar rats weighing about 300 g were fed a standard diet (Nihon Clea, CE-2) and provided tap water ad libitum.

Acute renal hypertensive rats: Normotensive male Wistar rats weighing about 300 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the trachea was cannulated with polyethylene tubing of an appropriate size. To cut off the left-side renal blood flow, a clamp was placed on the left renal pedicle, through a dorsal incision. Acute renal hypertension was produced by unclamping the left renal pedicle after it had been occluded for about 5 hours. Following release of the clamp, arterial blood pressure increased within 3 min and this increase in blood pressure was maintained for over one hour. YS-980 was given i.v. (1 mg/kg) when the blood pressure had attained a plateau level (Fig. 1) and the blood pressure was monitored continuously for one hour after the injection.

Two-kidney, one-clip hypertensive rats: Normotensive male Wistar rats weighing between 150 and 170 g were anesthetized with ether, and a silver clip (0.2 mm inside diameter) was placed on the left renal pedicle through a dorsal incision. The contralateral kidney was left intact. Following the operation, the rats were fed a standard diet and the blood pressure was measured by the tail plethysmographic method. After about 6 weeks, rats in which systolic blood pressure reached over 170 mmHg were used. The body weight was between 250 and 270 g.

Spontaneously hypertensive rats (SHR): About 20 weeks old male SHR weighing between 250 and 330 g were used. These rats were fed a standard diet and provided tap water ad libitum.

DOCA hypertensive rats: Normotensive male Wistar rats weighing between 150 and 170 g were anesthetized with ether and the left kidney was excised. These rats were given deoxycorticosterone acetate (DOCA), 15 mg/kg, s.c. once weekly. During this period, the animals were fed a standard diet which included 3% NaCl and tap water ad libitum. The blood pressure was measured intermittently following the first administration of DOCA. After about 9 weeks, rats in which systolic blood pressure reached over 170 mmHg were used for this experiment. The body weight was between 210 and 260 g.

Experimental protocol

Anesthetized rats: Normotensive and various hypertensive rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the blood pressure was monitored continuously through an indwelling polyethylene catheter (PE-50) in the left femoral artery using a Nihon Koden MP-4T pressure transducer in series with a recorder. Through a catheter introduced into the left femoral vein, these rats were given YS-980, 1 mg/kg (1 mg/ml in saline), i.v. followed by a flush of 0.3 ml of saline. As a control, 0.5 ml saline was injected. Blood pressure was monitored for 30 min after the injection of YS-980.

Conscious rats: An indwelling left femoral arterial catheter was implanted in normotensive and hypertensive rats, and the rats fixed on their back. This surgical procedure was performed under light ether anesthesia. After about 24 hours, the rats were housed in separate metabolic cages for the experimental period and blood pressure was recorded continuously from the indwelling catheter under conscious and unrestrained conditions. These rats were given oral administrations of a 5% tragacanth suspension of YS-980, 10 mg/kg (2 mg/ml), or vehicle (5%
tragacanth) alone as a control. Blood pressure was recorded for 2 hours after the YS-980 administration.

Statistical analysis
The paired comparison t-test was used for statistical analysis. A significance level of p<0.05 was used as the criterion of significance.

RESULTS

Anesthetized rats

Acute renal hypertensive rats: Figure 1 shows a typical pattern of hypotensive effect in an acute renal hypertensive rat. Following unclamping of the left renal pedicle which had been occluded for 5 hours, mean arterial pressure increased from 128.2±6.8 to 156.2±5.2 mmHg and stabilized at 155.6±4.6 mmHg in a few minutes. Intravenous administration of YS-980 markedly lowered mean arterial pressure to 100.8±8.1 mmHg (−54.8±7.3 mmHg) 3 min after injection, and to 118.4±4.6, 120.0±8.2 and 116.2±8.5 mmHg, 10, 30 and 60 min after injection, respectively (n=5). This significant hypotensive effect of YS-980 was long lasting and continued at least for one hour.

Normotensive and various hypertensive rats

![Graph](image_url)

Fig. 1. Hypotensive effect of YS-980 on acute renal hypertensive rats. Left renal pedicle was occluded with a clamp and after five hours the clamp was released. YS-980 (1 mg/kg) was given i.v. when the blood pressure increased and reached a plateau level.

![Graph](image_url)

Fig. 2. Hypotensive effect of YS-980 on anesthetized normotensive and various experimental hypertensive rats. The drug was given i.v. in a dose of 1 mg/kg. Each point represents mean±S.E. *: Significantly different from the blood pressure level before YS-980 injection (p<0.05).
rats (Fig. 2): In the anesthetized rats, control mean arterial blood pressures of normotensive, two-kidney, one-clip hypertensive, spontaneously hypertensive and DOCA hypertensive rats were 112.3±3.6 (n=6), 172.8±8.7 (n=8), 155.4±6.1 (n=7) and 145.8±3.0 (n=6) mmHg, respectively. A half milliliter of saline produced no significant changes in blood pressure in normotensive and various hypertensive rats. In normotensive rats, intravenous administration of YS-980 resulted in a slight decrease in blood pressure (~16.3±3.7 mmHg 3 min after injection) and this hypotensive effect continued for about 10 min.

In two-kidney, one-clip hypertensive rats, intravenous YS-980 administration markedly lowered mean arterial pressure to 133.0±11.4 mmHg (~39.8±5.9 mmHg) 3 min after injection and this hypotensive effect continued at least for 30 min (153.6±9.5 mmHg). YS-980 showed a moderate hypotensive effect on SHR. Intravenous YS-980 injection lowered mean arterial blood pressure to 126.2±9.3 mmHg (~29.2±4.4 mmHg) 3 min after injection, and this effect continued about 20 min. Contrary to these results, YS-980 produced no significant changes in blood pressure in DOCA hypertensive rats.

Conscious rats (Fig. 3)

In normotensive rats, oral administration of YS-980 resulted in a slight blood pressure decrease (9 mmHg at 30 min after administration), however this change in blood pressure was also observed by oral administration of the vehicle (5% tragacanth) alone. Similar to the results seen in the anesthetized rats, YS-980 markedly lowered the blood pressure from 184.6±4.6 to 133.6±9.8, 144.6±7.5 and 162.0±10.6 mmHg (n=5) 10 min, 1 and 2 hours after administration in two-kidney, one-clip hypertensive rats. In the control group, the mean arterial pressure before administration was 186.4±5.4 mmHg (n=5) and vehicle alone caused no significant changes in blood pressure over 1 hour.

In SHR, the mean arterial pressure was 187.4±7.4 mmHg (n=5). Ten minutes after YS-980 administration, blood pressure decreased to 171.2±9.5 mmHg, but this hypotensive effect was not so evident compared to that in the two-kidney, one-clip hypertensive rats.

Fig. 3. Hypotensive effect of YS-980 on conscious normotensive and various experimental hypertensive rats. The drug was given p.o. in a dose of 10 mg/kg. Each point represents mean±S.E. *: Significantly different from the blood pressure level before YS-980 administration (p<0.05). ○: Control, vehicle, 5 ml/kg, p.o. ●: YS-980, 10 mg/kg, p.o.
hypertensive rats. The maximum hypotensive effect (159.5±1.0 mmHg) was attained 80 min after administration. In the control group, the vehicle alone produced no significant changes in the blood pressure. YS-980 did not reduce the blood pressure in the DOCA hypertensive rats.

**DISCUSSION**

We have reported that YS-980 was the most potent orally effective CE inhibitor among various N-mercaptoacylamino acids derived from 2-mercaptopropionylglycine (tiopronin) in vitro and in vivo, and that the potency of this agent on the inhibitory effect of conversion from Al to All was considered to be similar to that of captopril (5, 6). Mita et al. (7) reported that YS-980 inhibited the conversion of Al to All as well as the inactivation of vasodilative bradykinin. Thus part of the antihypertensive action of this drug may be attributable to the effects on bradykinin potentiation.

The present study revealed that YS-980 markedly reduced the blood pressure in renin-angiotensin-dependent models of hypertension such as acute renal and two-kidney, one-clip hypertensive rats in both anesthetized and conscious conditions. The increase in plasma renin levels in acute renal hypertensive rats suggests that the renin-angiotensin system mediated the acute rise in blood pressure (9). It was also reported that an increase in renin activity is a contributory factor in the development and maintenance of renal hypertension in the acute phase of two-kidney, one-clip hypertensive rats (10, 11). Furthermore, in these two models of renal hypertension, pepstatin, a potent renin inhibitor (12), and All antagonist (10, 13) also lowered blood pressure markedly. The acute hypotensive effects of YS-980 in the renin-angiotensin-dependent models of hypertension may be the result of a decrease in circulating All levels via CE inhibition. However, plasma All levels remain to be assessed.

Contrary to these marked effects in renal hypertension, YS-980 moderately lowered the blood pressure in the established stage of SHR both in anesthetized and conscious animals. The involvement of the renin-angiotensin system in the pathogenesis and maintenance of high blood pressure in SHR is controversial. Plasma renin activity has been found to be low (14, 15), normal (16) and high (17) in the established stage of SHR. However, pepstatin (12), All antagonist (18) and angiotensin antibodies (19) had no significant effects on the blood pressure in SHR. Thus, the renin-angiotensin system is not always related to this model. It was also reported that urinary kallikrein excretion in 20 weeks SHR is significantly low as compared to their normotensive control strain (20). This finding suggests that an abnormality of the kallikrein-kinin system may be involved in the maintenance of such hypertension. Since YS-980 inhibits the inactivation of bradykinin as well as the conversion of Al to All, the antihypertensive effects of YS-980 in SHR may be the result of bradykinin potentiation.

In the present study, YS-980 did not reduce the blood pressure in DOCA hypertensive rats. Haack et al. (21) reported that the common mediator for blood pressure elevation due to DOCA excess would be an increase in extracellular fluid volume via renal sodium and water retention. Since the volume dependency is very high in this model of hypertension, YS-980, which affects mainly vasoactive humoral factors such as renin-angiotensin and kallikrein-kinin system, was not effective. On the other hand, in DOCA hypertensive rats, since the renin-angiotensin system is suppressed (21) and the kallikrein-kinin system is enhanced (22, 23) maximally by the repeated administration of DOCA, even if CE (kininase II) is inhibited by YS-
980, additional change in blood pressure via these humoral factors would be unlikely.

In normotensive rats, YS-980 had a slight but significant hypotensive action only in the anesthetized rats. Pettinger et al. (24) reported that plasma renin activity was significantly increased by sodium pento-barbital anesthesia in normal rats. Thus, the effect of an inhibitor of renin-angiotensin system would probably be greater under conditions of anesthesia.

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