Comparative Study of TA-606, a Novel Angiotensin II Receptor Antagonist, With Losartan in Terms of Species Difference and Orthostatic Hypotension

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ABSTRACT—Losartan is a prodrug type Angiotensin II (Ang II) AT₁-receptor antagonist whose efficacy depends on the oxidase activity of individuals. In addition, losartan affects the normal blood pressure and can potentially cause orthostatic hypotension. In this report, we examined effects of TA-606 (3-pentyloxy)carboxylxymethyl-5-acetyl-2-n-propyl-3-[2'(1H-tetrazole-5-yl)bibenyl-4-yl]methyl-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-4-carboxylate hydrochloride), a prodrug type AT₁-receptor antagonist, on the Ang II-induced pressor response and hypotension in a dog model, which is known to have lower oxidase activity than other species, and orthostatic hypotension in the rat tilting model. The results indicated that TA-606 was immediately converted to its active form, 606A, after oral administration, and it demonstrated potent inhibition of the Ang II-induced pressor response in conscious normotensive dogs (0.3–3 mg/kg, p.o.). It also had a potent hypotensive effect in conscious 2K,1C-renal hypertensive dogs (0.3–10 mg/kg, p.o.). These effects of TA-606 were 32 and 30 times more potent than those of losartan, respectively. In addition, EXP3174 (1, 10 mg/kg, i.v.), an active metabolite of losartan, but not 606A (1–30 mg/kg, i.v.) showed an orthostatic hypotensive effect in the rat tilting model. These results suggest that TA-606 is an effective Ang II receptor antagonist without the drawbacks of losartan.

Keywords: TA-606, Angiotensin II receptor antagonist, Conscious dog, Renal hypertension, Orthostatic hypotension

Several reports revealed that angiotensin II (Ang II) AT₁-receptor antagonists have advantages to angiotensin converting enzyme inhibitors (ACEIs), because Ang II generation is not completely inhibited by ACEIs (1) and ACEIs share common side effects; i.e., dry cough and angioedema (2). It is well accepted now that Ang II AT₁-receptor antagonists are useful agents for treatment of patients with hypertension and other cardiovascular diseases (3).

Losartan is a prototype of Ang II AT₁-receptor antagonists, and now it is used in the clinic (4). However, losartan is considered to have its own drawbacks; i.e., losartan is a prodrug type agent and its hypotensive effect depends on the generation of the active metabolite EXP3174 (5–7). Since generation of EXP3174 after oral administration of losartan depends on the oxidase activity of the individual, predictability of efficacy as well as side effects of losartan also depend on it (8, 9). Furthermore, it was reported that losartan lowered the normal blood pressure (10) and can potentially cause orthostatic hypotension (11).

The pharmacological characteristics of TA-606 ((3-pentyloxy)carboxylxymethyl-5-acetyl-2-n-propyl-3-[2'(1H-tetrazole-5-yl)bibenyl-4-yl]methyl-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-4-carboxylate hydrochloride) (Fig. 1) was already reported previously: TA-606 exhibits a potent hypotensive effect in two kidney, one clip (2K,1C)-renal hypertensive rats and spontaneously hypertensive rats (12). Its active metabolite, 606A, also exhibited a potent antihypertensive effect in spontaneously hypertensive rats (13) and induced regression of cardiac hypertrophy, augmented endothelium-dependent relaxation of the aorta and improved renal function in stroke-prone spontaneously hypertensive rats (14). Since TA-606 is the same prodrug type Ang II AT₁-receptor antagonist as losartan, it is possible that the amount of 606A generated after oral administration of TA-606 depends on the activity of metabolic enzymes, which may lead to low predictabilities of efficacy and side effects. Moreover, TA-606 might induce orthostatic hypotension like losartan.
The aim of the present study is to determine if the hypotensive effect of TA-606 is attenuated in dogs, which are known to possess lower oxidase activity than rats (8), and if it produces orthostatic hypotension in a rat tilting model. Through these investigations, we intend to clarify whether the drawbacks of losartan are the common features of Ang II AT₁-receptor antagonists or not.

MATERIALS AND METHODS

Animals and ethics

The animals used in the present study were obtained from following breeders: male mongrel dogs (12–20 kg), Oriental Yeast (Tokyo); male Sprague-Dawley rats (SD rats, Crl:CD, 8–10 weeks of age, 300–320 g), Charles River Japan (Yokohama). Our experiments were reviewed and approved by the committee on ethics of animal experiment at Tanabe Seiyaku Co., Ltd., which is in accordance with the guideline of The Japanese Pharmacological Society.

Measurements of arterial blood pressure and heart rate in dogs

In all conscious dog experiments, the telemetry method was used to measure arterial blood pressure and heart rate (HR). Under sodium pentobarbital anesthesia, a transmitter (TA11PA-D7; Primetech, Tokyo) was implanted subcutaneously at the lower back, and an attached cannula was inserted into the right femoral artery. The signal was received via a receiver (RM2000, Primetech) and amplified with a carrier amplifier (AP-621G; Nihon Kohden, Tokyo). HR was also measured by a cardiograph (AT-601G, Nihon Kohden) triggered by arterial pressure pulses. Mean arterial blood pressure (MAP) and HR were simultaneously recorded on a linear recorder (WR-3300; Graphtech, Tokyo) and also stored in a computer system (Acqknowledge; Physiotech, Tokyo). MAP and HR were estimated by average values of 60-s data at each time point.

Experiment of antagonistic action on Ang II-induced pressor response

Ang II, 0.1 μg/kg, was intravenously injected into the forearm vein of the instrumented dog, which was trained to lie down on a table, at 45, 30 and 15 min before and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 420 min after oral administration of the test drug. Ang II-induced pressor response was expressed as a percent of that obtained just before the administration of test drug. Dogs were used repeatedly. At least a week was allowed to pass...
between the experiments.

Experiment in renal hypertensive dogs (2K,1C)

Dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and the left renal artery was exposed through a retroperitoneal flank incision. The flow probe of an electromagnetic flowmeter (MFV-3100, Nihon Kohden) was placed around the artery, and the renal artery was constricted to reduce the blood flow to 30% to 40% of baseline value (134±20 ml/min to 45±10 ml/min) with an adjustable clamp. The incision was sutured and an antibiotic (Dopen®, Glaxo, Middlesex, UK) was given.

Experiments were done on the established phase of hypertension (>150 mmHg of systolic blood pressure). Dogs were placed in home cages, and arterial blood pressure and HR were continuously measured for at least 26 h by a telemetry method as described. After an observation period of 2 h or more, test drugs were administered orally. Dogs were used repeatedly. At least a week was placed between the experiments.

Tilting test

Rats were anesthetized with thiobutabarbitonal (100 mg/kg, i.p.). In all experiments, arterial blood pressure was measured with a pressure transducer (TP-400T, Nihon Kohden) connected to a polyethylene cannula, which was inserted into the right femoral artery. HR was also measured by a cardiotachometer (AT-601G, Nihon Kohden) triggered by arterial blood pressure pulses. Arterial blood pressure and HR were simultaneously recorded on a linear recorder (WR-3300, Graphitech). A venous cannula for drug injection was inserted into the left femoral vein.

The tilt experiment (a test of postural hypotension) was conducted by raising the head side of a tilt board from a supine to a 45° head-up position for 1 min. The pressure transducer was maintained at the level of the heart so that tilting does not influence blood pressure measurement. After a stabilization period for blood pressure and HR and a training period consisting of 2 or 3 tilts at intervals of 10 min, data collection was started. Control tilts consisted of two determinations were made at an interval of 15 min prior to drug or vehicle (saline) administration. Fifteen minutes after the second control tilt, animals were dosed intravenously with saline, 606A, EXP3174 or prazosin. Then, the tilting test was repeated at 15, 30, 45, 60, 90 and 120 min after dosing.

Measurement of plasma concentration of 606A

Plasma concentration of 606A was determined by high-performance liquid chromatography with an ultraviolet detector set at 254 nm (model 484; Waters Assoc., Milford, MA, USA) using normal conscious dogs as described by the previous report (12). TA-606, 1 mg/kg, was administered orally to dogs and blood was collected from the forearm vein at 0.5, 1, 2, 4, 7 and 24 h after the administration. The blood was then centrifuged to separate its plasma. We also examined the presence of metabolites other than 606A in the plasma.

Drugs

In dog experiments, test drugs were packed in hard gelatin capsules and given orally with tap water (20 ml). The dog was deprived of food for 16 h before drug administration. In the tilting test, all compounds were dissolved in saline at a volume of 0.1 ml/100 g body weight. TA-606, 606A, losartan and EXP3174 were synthesized at Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd. (Saitama). Prazosin hydrochloride was obtained from Sigma (St. Louis, MO, USA). Other chemicals with the highest grade were purchased commercially.

Data analysis

All data were expressed as means±S.E.M. Statistical analysis for the experiment in conscious dogs and basal values of tilting test was performed by repeated measures analysis of variance (ANOVA) with the Bonferroni correction. In the tilting test, comparison was made by analysis of variance followed by the t-test or paired t-test alone when appropriate. Differences at P<0.05 were considered to be statistically significant.

RESULTS

Antagonistic action on Ang II-induced pressor response in conscious normotensive dogs

Basal MAP and HR of our conscious normotensive dogs were 80.9±6.0 mmHg and 76.1±5.5 beats/min, respectively (n=4). Ang II-induced a pressor response of 53.3±4.0 mmHg in conscious dogs. As shown in Fig. 2A, TA-606 (0.3, 1, 3 mg/kg, p.o.) dose-dependently inhibited the Ang II-induced pressor response. The inhibitory effect of TA-606 on Ang II-induced pressor response was statistically significant until 5 h after dosing at a dose of 3 mg/kg, p.o. On the other hand, as shown in Fig. 2B, the inhibitory effect of losartan on the Ang II-induced pressor response was weaker and shorter lasting than that of TA-606. The inhibitory effect of 30 mg/kg, p.o. losartan, which caused a comparable inhibition of the Ang II-induced pressor response to TA-606 at 3 mg/kg, p.o., lasted only 3 h. TA-606 did not influence the basal blood pressure (0.3, 1, 3 mg/kg, p.o.), although 30 mg/kg, p.o. losartan significantly lowered basal blood pressure (Fig. 2, middle panel). Neither drug affected the HR in this model (Fig. 2, lower panel).
**Hypotensive effect of TA-606 in conscious renal hypertensive dogs**

After establishment of hypertension, we started the experiment. As shown in the vehicle group of Fig. 3, we could observe stable MAP and HR in conscious non-restricted dogs under our experimental condition. Deviation of the blood pressure was within approximately ±5 mmHg in our experiment.

TA-606 dose-dependently lowered blood pressure with rapid onset as shown in Fig. 3A. The peak hypotensive effect of TA-606 appeared 2 to 4 h after dosing. The hypotensive effect of TA-606 (0.3 to 3 mg/kg, p.o.) recovered within 16 h, but the hypotensive effect of TA-606 (10 mg/kg, p.o.) lasted over 24 h (Fig. 3A).

Losartan also lowered blood pressure dose-dependently, but was approximately 30 times less potent than TA-606. Even at a dose of 30 mg/kg, p.o., the hypotensive effect of losartan did not last more than 10 h in this model (Fig. 3B).

Neither drug affected the HR in this model (Fig. 3, lower panel).

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**Fig. 2.** Inhibitory effects of TA-606 (A) (vehicle: open circle, 0.3 mg/kg: solid triangle, 1 mg/kg: solid circle, 3 mg/kg: solid square) and losartan (B) (vehicle: open circle, 10 mg/kg: solid triangle, 30 mg/kg: solid square) on Ang II-induced pressor response (upper panel), basal blood pressure (middle panel) and heart rate (lower panel) in conscious normotensive dogs. Symbols and vertical bars represent means±S.E.M. of 4 experiments.
Fig. 3. Effects of TA-606 (A) (vehicle: open circle, 0.3 mg/kg; solid triangle, 1 mg/kg; solid circle, 3 mg/kg; solid square, 10 mg/kg; small square) and losartan (B) (vehicle: open circle, 10 mg/kg; solid triangle, 30 mg/kg; solid square) on mean arterial pressure and heart rate in 2K,1C-renal hypertensive dogs. Each point and vertical bar indicates the mean±S.E.M. of 4 experiments.

Fig. 4. The time course of suppressive action on Ang II-induced pressor response (A) or hypotensive (B) effect of TA-606 (solid circle) and plasma concentration of 606A (open circle). Each point and vertical bar indicates the mean±S.E.M. of 4 experiments.
Relationship between antagonistic action on pressor response to Ang II and hypotensive effect of TA-606, and plasma concentration of 606A

We did not find any signs of the presence of TA-606 or its metabolites except for 606A in normal dog plasma. The plasma concentration of 606A is plotted with the antagonistic effect of TA-606 on pressor response to Ang II (Fig. 4A) and with the hypotensive effect of TA-606 (Fig. 4B). As shown in these figures, an inhibitory effect of TA-606 on Ang II-induced pressor response moderately correlated with the plasma concentration (correlation factor \(r^2\)=0.80). However, the hypotensive effect of TA-606 did not correlate with the plasma concentration \(r^2=0.02\). The hypotensive effect of TA-606 in conscious 2K,1C-renal hypertensive dogs was still observed even if the plasma concentration decreased below the detection limit (<1 ng/ml).

Effect of 606A and EXP3174 on postural change-induced hypotension in normotensive rats

Previously, we have reported that IC\(_{50}\) values of 606A

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mmHg) before</th>
<th>MAP (mmHg) after</th>
<th>HR (beats/min) before</th>
<th>HR (beats/min) after</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>97.2±1.8</td>
<td>96.3±1.5</td>
<td>316.0±5.2</td>
<td>314.3±9.7</td>
</tr>
<tr>
<td>Prazosin, 0.1 mg/kg</td>
<td>95.2±4.7</td>
<td>72.4±1.1**</td>
<td>319.8±13.9</td>
<td>345.8±19.6</td>
</tr>
<tr>
<td>606A, 1 mg/kg</td>
<td>95.5±2.5</td>
<td>92.3±5.1</td>
<td>316.8±9.7</td>
<td>318.3±8.4</td>
</tr>
<tr>
<td>606A, 10 mg/kg</td>
<td>94.8±2.7</td>
<td>90.3±5.4</td>
<td>313.8±11.7</td>
<td>317.3±5.4</td>
</tr>
<tr>
<td>606A, 30 mg/kg</td>
<td>94.5±2.0</td>
<td>89.5±6.6</td>
<td>315.0±6.2</td>
<td>320.5±12.7</td>
</tr>
<tr>
<td>EXP3174, 1 mg/kg</td>
<td>92.2±2.3</td>
<td>87.3±2.9</td>
<td>318.0±7.2</td>
<td>322.0±18.0</td>
</tr>
<tr>
<td>EXP3174, 10 mg/kg</td>
<td>94.7±3.5</td>
<td>83.5±2.1*</td>
<td>317.5±7.3</td>
<td>321.0±7.6</td>
</tr>
</tbody>
</table>

MAP and HR were determined before and 30 min after the intravenous administration of test compounds. Values are means ± S.E.M. of 4–6 experiments. *P<0.05 and **P<0.01: significantly different from the corresponding value before drug administration.

Fig. 5. Effect of vehicle (A) and prazosin (0.1 mg/kg, i.v.) (B) on 60 ± 45° head-up tilt test (orthostatic hypotension) at 30 min after dosing. Upper panel and lower panel represent the changes in blood pressure and heart rate, respectively. Open and closed circles indicate before drug administration and 30 min after drug administration, respectively. Each point and vertical bar indicates the mean ± S.E.M. of 6 experiments. *P<0.05, **P<0.01, ***P<0.001: significantly different from control values.
and EXP3174 on Ang II (0.1 μg/kg, i.v.)-induced pressor response in anesthetized rats were 6 μg/kg, i.v. and 50 μg/kg, i.v., respectively (12); and doses of ≥0.1 mg/kg, i.v. 606A and ≥1 mg/kg, i.v. EXP3174 were enough to cause ≥90% inhibition of the action of Ang II. Therefore, we evaluated the effect of sufficient doses of 606A and EXP3174 to inhibit the Ang II-induced pressor response on orthostatic hypotension in this experiment.

Basal MAP and HR in the tilting test are shown in Table 1. Prazosin, 0.1 mg/kg, i.v., significantly lowered basal MAP and tended to increase basal HR. EXP3174, 10 mg/kg, i.v., also significantly lowered basal MAP, but 606A did not show any significant effects even at the highest dose of 30 mg/kg, i.v.

As shown in Fig. 5A, head-up tilt induced short-lasting drop of blood pressure initially and continuous elevation of HR. These changes observed in the tilting test were not altered after vehicle treatment.

Figure 6 shows the time courses for the effects of prazosin, 606A and EXP3174 on the tilting-induced changes in MAP and HR when they are evaluated at 20 s after the start of each tilting. Animals treated with prazosin, 0.1 mg/kg, i.v., which we used as a reference compound in this model, showed significantly larger hypotension and less tachycardia than those in the vehicle group; i.e., orthostatic hypotension (Figs. 5 and 6). At doses of 1, 10 and 30 mg/kg, i.v., 606A did not show any orthostatic hypotensive effect during our experiment (Figs. 6 and 7). On the other hand, EXP3174 at a dose of 10 mg/kg, i.v. caused orthostatic hypotension. The peak response to EXP3174 was observed 30 min after the drug administration (Figs. 6 and 8).

![Graph showing changes in mean arterial pressure and heart rate over time](image)

**Fig. 6.** Effect of vehicle, 606A (1, 10, 30 mg/kg, i.v.), EXP3174 (1, 10 mg/kg, i.v.) and prazosin (0.1 mg/kg, i.v.) on orthostatic hypotension at 20 s after the onset of each 45° head-up tilt test. Upper and lower panel represent changes in blood pressure and heart rate, respectively. Each value and vertical bar indicates the mean±S.E.M. of 4–6 experiments. *P<0.05, **P<0.01: significantly different from the vehicle group.
DISCUSSION

We have previously reported that the values of $ED_{50}$ (30 mmHg hypotensive dose) of TA-606 and losartan, the dose at which each compound induces half maximal hypotensive effect, were 0.14 mg/kg, p.o. and 4.2 mg/kg, p.o. in conscious 2K,1C-renal hypertensive rats, respectively (12). On the other hand, $ED_{15}$ (15 mmHg hypotensive dose) of TA-606 and losartan in conscious 2K,1C-renal hypertensive dogs were found to be 0.45 mg/kg, p.o. and 26 mg/kg, p.o., respectively, since $ED_{15}$, which we used in this report, is also the dose that induces the half maximal hypotensive effect in renal hypertensive dogs. Therefore, we could demonstrate that the difference between effective doses of TA-606 in rats and dogs was much smaller than that of losartan.

Generation of EXP3174 after oral administration of losartan depends on the oxidase activity of individuals (6, 7). Therefore, it may be difficult to predict not only efficacy but also side effects of losartan in the clinic. TA-606 exhibited comparable efficacy in both rat and dog renal hypertensive models, although TA-606 is a prodrug type Ang II AT$_1$-receptor antagonist like losartan. Thus, we showed that it is easier to predict the efficacy and side effects of TA-606 than those of losartan in the clinic.

It is also important to clarify whether generation of other metabolites could occur after oral administration of TA-606. We did not find any metabolites of TA-606 other than 606A in the plasma of dogs. It was already confirmed that the only TA-606 metabolite in rat plasma is 606A (12). These observations also assure the high predictability of TA-606.

Plasma concentration of 606A reached its peak level at 1 h after administration to normotensive dogs, whereas the peak responses of the suppressive action on Ang II-induced pressor response and hypotensive effect were achieved at 1.5 and 2 h after dosing, respectively. Thereafter, 606A disappeared rapidly from the plasma in spite of a gradual decline of its pharmacological effects. It was already reported that the hypotensive effect of an AT$_1$-receptor antagonist was in parallel with its receptor occupancy rather than plasma concentration (15). Although renal excretion of a drug might be influenced by constriction of the renal artery and thus clearance of the drug.
might be delayed, it can be assumed that TA-606 rapidly reaches the Ang II AT₁ receptor after the conversion to its active form, but its dissociation from the receptor is slow. Previously it was reported that 606A showed insurmountable antagonism in the isolated guinea pig aorta (13). Antagonistic manner of 606A supports low dissociation rate from the receptor.

Losartan significantly reduced basal blood pressure in conscious normotensive dogs. Previously we have reported that losartan lowered blood pressure in deoxycorticosterone acetate-salt hypertensive models (12). Dowell et al. also reported that chronic oral treatment of normotensive rats with losartan for 3 weeks at a dose of 10 mg/kg per day reduced their blood pressure significantly (16). Moreover, intravenous administration of losartan (10 mg/kg per day) to normal rats for 10 days suppressed endogenous Ang II and lowered the blood pressure (17). One of the possible mechanisms of these effects is that losartan may inhibit glutamate receptor binding and may influence the normal blood pressure and its regulatory system (18). Glutamate has been reported to be a sympatoexcitatory amino acid in the central nervous system, and losartan inhibited the sympatoexcitatory response evoked by l-glutamate, whereas [Sar¹, Thr⁴]-Ang II did not inhibit the sympathoexcitatory response evoked by l-glutamate (10). Therefore, losartan may possess a mechanism other than AT₁ blockade. It was also reported that losartan at a dose of 10 mg/kg, i.d. produced marked orthostatic hypotension in renin-dependent hypertensive rats (19).

Since TA-606 is a prodrug type Ang II AT₁-receptor antagonist like losartan, TA-606 might induce orthostatic hypotension. The initial drop of the blood pressure on tilting is due to the inability of the baroreflex system to counteract against the abrupt change in posture which results in pooling of blood in the lower parts of the body (20). Prazosin, a selective α₁-blocker, is known to cause orthostatic hypotension during its initial therapy for hypertension (21). Since prazosin enhanced the tilting-induced hypotension in our study, we considered that our experimental rat tilting model was proper to evaluate the possibility to cause postural hypotension. Although we did not recognize the possibility of 606A to cause postural hypotension, EXP3174 induced orthostatic hypotension like prazosin.

As various AT₁-receptor antagonists are developed, it
seems very important to determine whether individual AT$_1$-receptor antagonist influence the normal blood pressure regulatory system.

Our results in the present study suggest that the species difference of TA-606 is smaller than that of losartan. TA-606 did not show orthostatic hypotensive action even though TA-606 is an Ang II AT$_1$-receptor antagonist like losartan. Therefore, all of the prodrug type Ang II AT$_1$-receptor antagonists do not necessarily show species differences and the potential for inducing orthostatic hypotension. We must investigate these possibilities of prodrug type Ang II AT$_1$-receptor antagonists individually.

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