Cardiovascular Effects of 1,5-Benzothiazepine Derivatives Having a l-cis and d-cis Configuration in Anesthetized Dogs

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TA-993, a new 1,5-benzothiazepine derivative having a l-cis configuration, has a selective increasing action on limb blood flow, in addition to an antplatelet aggregating action. The cardiovascular action of TA-993 is quite different from diltiazem, which is a well-known 1,5-benzothiazepine derivative having a d-cis configuration. Therefore, we compared the cardiovascular actions of d-cis and l-cis isomers of TA-993 with those of diltiazem. l-cis-Diltiazem, as well as TA-993, progressively increased femoral, brachial and common carotid blood flow with little change in arterial pressure or vertebral blood flow. However, the peak response to l-cis-diltiazem (20 min after the administration) was observed earlier than that to TA-993 (60 min after the administration). On the other hand, d-cis-TA-993, as well as diltiazem, caused transient hypotension, tachycardia and increases in vertebral, brachial, femoral and common carotid blood flow. Furthermore, their peak effects were observed immediately after the administration. Potency ratios of the vasorelaxing effects of TA-993, l-cis-diltiazem and d-cis-TA-993 to diltiazem in the isolated and K+ contracted canine femoral artery were 0.096, 0.032 and 1.209, respectively. pA₂ values for TA-993 and diltiazem against Ca²⁺ induced contractions in the isolated and K⁺ depolarized canine saphenous artery were 5.50±0.11 and 7.12±0.18, respectively. These results indicate that TA-993 shares a common profile with l-cis-diltiazem, and suggest that the 1,5-benzothiazepine derivatives of a l-cis configuration are a different class of drug from that of the d-cis configuration.

Key words 1,5-benzothiazepine derivative; optical isomer; cardiovascular effect; TA-993; diltiazem

Diltiazem, a well known 1,5-benzothiazepine calcium antagonist, has potent spasmolytic and vasodilating action on coronary and vertebral arteries, as well as hypotensive action. Since 1,5-benzothiazepine derivatives have two asymmetric carbon atoms, there can be 4 isomers. d-cis isomers of this chemical group, such as diltiazem and clentiazem, generally have a potent blocking action on voltage-dependent L-type Ca channels, which is the mechanism of their cardiovascular effect.

On the other hand, the other isomers, i.e., l-cis, d-trans and l-trans isomers, of 1,5-benzothiazepine derivatives, have been reported to have only weak cardiovascular effects because of their much less potent calcium blocking action in comparison to those of d-cis isomers. However, we have found in a previous study that TA-993, (−)−cis-3-acetoxy-5-(2-(dimethylamino)ethyl)-2,3-dihydro-8-methyl-2-(4-methylphenyl)-1,5-benzothiazepin-4(SH)-one maleate, a new 1,5-benzothiazepine derivative having a l-cis configuration (Fig. 1), has a potent and unique increasing action on limb blood flow, in addition to an inhibitory effect on platelet aggregation. Since the cardiovascular effect of TA-993 is quite different from that of diltiazem, we hypothesized that the difference in cardiovascular effect between TA-993 and diltiazem would be mainly due to the difference in their optical configurations, i.e., l-cis and d-cis configurations.

In the present study, we compared the cardiovascular effect of TA-993 with diltiazem and their optical isomers, i.e., d-cis-TA-993 and l-cis-diltiazem, in anesthetized dogs, for the purpose of verifying our hypothesis.

MATERIALS AND METHODS

Cardiovascular Effects in Anesthetized Dogs Twelve mongrel dogs of either sex, weighing 11.5—18.0 kg, were anesthetized with an intravenous administration of sodium pentobarbital (30—35 mg/kg and 5.0—5.5 mg/kg/h), then placed on a heated operating table in the supine position. The trachea was intubated and artificially ventilated (15 ml/kg/ stroke, 20 strokes/min) with room air. Arterial blood pressure was measured with a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan) connected to a polyethylene catheter, inserted into the right brachial artery, and a carrier amplifier (AP-621G, Nihon Kohden, Tokyo, Japan). Heart rate was measured with a heart rate counter (AT-610G, Nihon Kohden, Tokyo, Japan) triggered by arterial pressure pulses. The left common carotid, right vertebral, left brachial and right femoral arteries were exposed. Flow probes (2.0—3.5 mm in inner diameter) connected to electromagnetic flowmeters (MFV-2100, Nihon Kohden, Tokyo, Japan) were placed on them to measure their blood flow. All measurements were simultaneously recorded on a multi-channel thermal pen-recorder (WR3310, Graphtec, Tokyo, Japan). Three groups of dogs (4 dogs each) were prepared because the cardiovascular effects of TA-993 and l-cis-diltiazem were quite long-lasting, as described later. In one group, diltiazem (10, 30, 100 μg/kg, i.v.), d-cis-TA-993 (10, 30, 100 μg/kg, i.v.) and l-cis-diltiazem (100 μg/kg, i.v.) were administered intravenously in this order. The administration of each dose of each drug was performed after the effect of the previous ad-

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ministration disappeared. In another group, only TA-993 (100 µg/kg) was administered intravenously. The last group served as a control, in which the same volume of vehicle was administered intravenously.

Studies in the Isolated Canine Arteries Nineteen mongrel dogs of either sex, weighing 8.7—21.0 kg, were anesthetized with an intravenous administration of sodium pentobarbital (30 mg/kg) and were euthanized by the intravenous administration of saturated potassium chloride (KCl). The femoral or saphenous arteries (approximately 3 and 1 mm in outer diameter, respectively) were dissected immediately, and arterial ring segments of 5 mm in length were prepared free from surrounding connective tissue in cooled physiological salt solution (PSS) of the following composition (mm): NaCl 118, KCl 4.7, CaCl₂ 2.55, MgSO₄ 1.18, KH₂PO₄ 1.18, NaHCO₃ 24.88, glucose 11.1, pH 7.3—7.4. The PSS was gassed with 95% O₂—5% CO₂ beforehand. Great care was taken not to touch the intimal surface. Some of the arteries with surrounding connective tissue were immersed in PSS at 4°C and stored overnight, and arterial segments were prepared as described above on the next day.

A. Vasorelaxing Effects in the Isolated Canine Femoral Artery Contracted with 40 mm K⁺. The ring segments of the femoral artery were suspended in organ baths (10 ml) filled with PSS, which were maintained at 37.0 ± 0.5°C and continuously gassed with 95% O₂—5% CO₂. Isometric tension was measured with a strain gauge transducer (UL-10GR, Minebea, Tokyo, Japan) and recorded on a multi-channel pen-recorder (MC6625A, Graphtec, Tokyo, Japan). The resting tension was adjusted to 1.0 ± 0.2 g. The arterial segments were allowed to equilibrate for 90—120 min. The solution in the organ baths was changed every 20—25 min during the equilibration period. After the equilibration period, the concentration of potassium ions (K⁺) in the baths was changed to 40 mm by adding 100 µl of 3.5 m KCl solution. After the tonic contraction was stabilized, drugs were cumulatively added to the bathing medium. At the end of each experiment, 10⁻⁴ M of papaverine was added to induce complete relaxation of the preparation.

B. Inhibitory Effects on a Ca²⁺-Induced Contraction in the Isolated Canine Saphenous Artery. The ring segments of the saphenous artery were suspended in organ baths (10 ml) filled with normal HEPES buffer solution, which was maintained at 37.0 ± 0.5°C and continuously gassed with 100% oxygen. The composition of normal HEPES buffer solution was as follows (mm): NaCl 140, KCl 5.0, CaCl₂ 2.5, MgCl₂ 1.0, HEPES 5.0, glucose 10.0. The pH of the solution was adjusted to 7.4 at 37°C by adding 1 N NaOH to the solution. Recording of the isometric tension was performed as described. After the equilibration period, 40 mm K⁺-contraction was observed to confirm the viability of the segment. After that, the solution in the baths was changed to Ca²⁺-free HEPES buffer solution, of which the composition is the same as normal HEPES buffer solution, except for the absence of CaCl₂. The Ca²⁺-free HEPES buffer solution in the baths was changed 2—3 times at an interval of 20—25 min. Then, the concentration of K⁺ in the baths was raised to 40 mm again. After the stabilization, concentration-response curves for Ca²⁺ were obtained by adding a CaCl₂ solution of various concentrations cumulatively. The procedure was repeated to obtain the second curve, either in the absence or presence of TA-993 or diltiazem. Each drug was added to the bathing medium 60 min before the determination of the second concentration—response curve for Ca²⁺.

Animal Ethics Our study was carried out in accordance with the declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Drugs TA-993, diltiazem hydrochloride, d-cis-TA-993, and l-cis-diltiazem hydrochloride were obtained from Tanabe Seiyaku Co., Ltd. (Osaka, Japan). Sodium pentobarbital and papaverine hydrochloride were obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). The other drugs used were of the best quality commercially available.

Drugs were dissolved in 0.9% sodium chloride solution and administered intravenously in a volume of 0.1 ml/kg in anesthetized dogs.

In the experiment involving isolated canine arteries, drugs were dissolved in distilled water and were added to the bathing medium in a volume of 100 µl.

Doses of all drugs were expressed in terms of the salt.

Statistical Analysis Values were represented as means ± S.E.M.

The time course data of cardiovascular effects of TA-993 and l-cis-diltiazem, which were expressed in the percentage change from the baseline value, were analyzed by repeated measures analysis of variance, and when the interaction between the drug and the time course was significant, the time course data of each drug was analyzed by repeated measures analysis of variance using Bonferroni's adjustment in the α level for comparison with the control experiment.

In the isolated canine femoral artery contracted with 40 mm K⁺, relaxation induced by papaverine (10⁻⁴ M) was taken as 100%, and EC₅₀, which represents the concentration causing 50% relaxation, was calculated with 4-parametters logistic function curves by the Simplex method, or by the Damping Gauss-Newton method in an individual segment.

The Ca²⁺-induced contraction in the isolated canine saphenous artery in the presence of TA-993, diltiazem or vehicle was expressed as a percentage of the maximal contraction of the first series of Ca²⁺-induced contractions. The EC₅₀ value for the Ca²⁺-induced contraction in the presence of each drug was calculated in individual segments as described above. The pA₂ values for TA-993 and diltiazem were calculated according to the method of Arunlakshana and Schild.¹⁷

RESULTS

Cardiovascular Effects of TA-993, Diltiazem and Their Optical Isomers in Anesthetized Dogs Figure 2 shows the time course of the increasing effect of TA-993 (100 µg/kg, i.v.) on the blood flow of common carotid, vertebral, brachial and femoral arteries in anesthetized dogs. TA-993 gradually but obviously increased the blood flow of common carotid, brachial and femoral arteries (p<0.01, vs. the control experiment). The peak responses were observed at about 60 min after the administration, and thereafter the increased blood flow remained at a peak level until 300 min after the administration. TA-993 did not cause any significant change in mean arterial pressure, heart rate or vertebral blood flow.

l-cis-Diltiazem (100 µg/kg, i.v.) also progressively in-
Fig. 2. Increasing Action of TA-993 on Common Carotid, Brachial and Femoral Blood Flow in Anesthetized Dogs

The symbols are as follows: TA-993 100 μg/kg, i.v. (●), control (vehicle i.v.) (○). Each point and vertical bar represents the mean±S.E.M. of 4 experiments. Baseline values in the TA-993-administered group and the control group are as follows: mean arterial pressure: 126.8±4.6 mmHg and 127.0±4.7 mmHg; heart rate: 138.0±4.1 beats/min and 143.3±6.8 beats/min; common carotid blood flow: 171.3±49.3 ml/min and 172.0±49.5 ml/min; vertebral blood flow: 24.3±1.9 ml/min and 22.0±0.8 ml/min; brachial blood flow: 66.8±20.9 ml/min and 74.5±29.3 ml/min; femoral blood flow: 79.8±22.2 ml/min and 87.8±39.6 ml/min. *p<0.01 vs. control (repeated measures analysis of variance using Bonferroni’s adjustment in the α level).

Fig. 3. Cardiovascular Effect of l-cis-Diltiazem in Anesthetized Dogs

The symbols are as follows: l-cis-diltiazem 100 μg/kg, i.v. (●), control (vehicle i.v.) (○). Each point and vertical bar represents the mean±S.E.M. of 4 experiments. Control data are reproduced from Fig. 2. Baseline values in the l-cis-diltiazem-administered group are as follows: mean arterial pressure: 111.8±9.7 mmHg; heart rate: 125.5±11.9 beats/min; common carotid blood flow: 137.5±25.6 ml/min; vertebral blood flow: 27.5±1.6 ml/min; brachial blood flow: 59.3±9.4 ml/min; femoral blood flow: 50.3±10.4 ml/min. *p<0.01 vs. control (repeated measures analysis of variance using Bonferroni’s adjustment in the α level).

creased the blood flow of the common carotid, brachial and femoral arteries (*p<0.01, vs. the control experiment) with little change in mean arterial pressure or vertebral blood flow. The blood flow reached peak responses 20–30 min after the administration. The increasing effect on the blood flow was sustained for 60 min after the administration, although the increased blood flow gradually decreased after the peak. In addition, l-cis-diltiazem slightly but persistently increased the heart rate as compared with the control group (*p<0.01) (Fig. 3).

On the other hand, d-cis-TA-993 (10, 30, 100 μg/kg, i.v.) and diltiazem (10, 30, 100 μg/kg, i.v.) both caused dose-de-
Fig. 4. Cardiovascular Effect of d-cis-TA-993 in Anesthetized Dogs

The symbols are as follows: d-cis-TA-993 10 μg/kg (○), 30 μg/kg (△), 100 μg/kg, i.v. (■). Each point and vertical bar represents the mean±S.E.M. of 4 experiments. Baseline values are as follows: mean arterial pressure: 107.3±10.5 mmHg; heart rate: 116.0±9.8 beats/min; common carotid blood flow: 146.3±20.8 ml/min; vertebral blood flow: 29.5±1.7 ml/min; brachial blood flow: 44.8±11.2 ml/min; femoral blood flow: 57.3±8.1 ml/min.

Fig. 5. Cardiovascular Effect of Diltiazem in Anesthetized Dogs

The symbols are as follows: diltiazem 10 μg/kg (○), 30 μg/kg (△), 100 μg/kg, i.v. (■). Each point and vertical bar represents the mean±S.E.M. of 4 experiments. Baseline values are as follows: mean arterial pressure: 105.2±8.7 mmHg; heart rate: 118.5±11.8 beats/min; common carotid blood flow: 173.8±19.2 ml/min; vertebral blood flow: 32.3±2.9 ml/min; brachial blood flow: 52.5±15.6 ml/min; femoral blood flow: 70.0±8.4 ml/min.

Pendent hypotension, tachycardia, a marked increase in vertebral blood flow, and moderate increases in femoral, brachial and common carotid blood flow. These effects appeared immediately after the administration, and almost disappeared within 30 min, except the effect of d-cis-TA-993 on vertebral blood flow (Figs. 4, 5).
artery at $10^{-7}$ M or more. On the other hand, TA-993 and l-cis-diltiazem also caused concentration-dependent relaxation with much less potency. Considering $EC_{30}$ values, the vasorelaxing effects of TA-993 and l-cis-diltiazem were approximately 10 and 30 times less potent than that of diltiazem, respectively, while the vasorelaxing effect of d-cis-TA-993 was as potent as that of diltiazem (Table 1).

**Antagonistic Activity of TA-993 and Diltiazem on Ca$^{2+}$-Induced Contractions in the Isolated Canine Saphenous Artery** Figures 7A and B show concentration-response curves for a Ca$^{2+}$-induced contraction with or without pretreatment with TA-993 or diltiazem, respectively. One or 3 mM of Ca$^{2+}$ induced the maximal contraction (maximal developed tension: $425 \pm 0.17$ g, $n=48$) in the absence of either TA-993 or diltiazem. Pretreatment with TA-993 (3 x $10^{-6}$—3 x $10^{-5}$ M) or diltiazem (3 x $10^{-6}$—3 x $10^{-7}$ M) caused a concentration-dependent rightward shift of the concentration-response curves for Ca$^{2+}$. The slopes of the Shild plot for the antagonistic activity of TA-993 and diltiazem against Ca$^{2+}$ were 1.34 ± 0.24 and 0.86 ± 0.36, respectively, which were not significantly different from 1.00 by the one-sample t-test (TA-993: $t=1.41$, $p(95%)=0.20$; diltiazem: $t=0.39$, $p(95%)=2.16$). $pA_{2}$ values for TA-993 and diltiazem against Ca$^{2+}$ were 5.50 ± 0.11 and 7.12 ± 0.18, respectively.

**DISCUSSION**

In the present study, we investigated the cardiovascular action of d-cis and l-cis isomers of TA-993 and diltiazem, both of which are 1,5-benzothiazepine derivatives, to verify our hypothesis as follows: The differences in cardiovascular effects between TA-993 and diltiazem are due mainly to differences in their optical configurations, i.e., l-cis and d-cis configurations.

l-cis-Diltiazem, as well as TA-993, both l-cis isomers, showed an increasing action on femoral, brachial and common carotid blood flow, while they did not cause any significant change in mean arterial pressure. The increasing action of l-cis-diltiazem on these blood flows developed slowly, like that of TA-993, but the peak effect of l-cis-diltiazem was observed earlier than that of TA-993. Although l-cis-diltiazem persistently increased heart rate, the increase was very slight. Thus, l-cis-diltiazem showed similar cardiovascular action to TA-993. The reported pharmacological actions of l-cis-diltiazem include a protective effect on the myocardial damage induced by ischemia and reperfusion, and a blocking action on the light-regulated current in rod photoreceptors as well as weak calcium antagonism, a weak and transient cardiovascular effect and an inhibitory effect on...
platelet aggregation. Therefore, this is the first report that l-cis-diltiazem shows the potent and unique cardiovascular effect we described.

As described above, the blood flow-increasing action of l-cis-diltiazem, as well as that of TA-993, in anesthetized dogs, was highly selective to certain vascular beds, i.e., femoral, brachial and common carotid arteries. Since the regions governed by these arteries are mainly skin and skeletal muscle, l-cis-diltiazem and TA-993 both are thought to increase skin and/or skeletal muscle blood flow. Thus, l-cis isomers of 1,5-benzothiazepine derivatives are suggested to selectively increase skin and/or muscle blood flow.

In contrast, d-cis-TA-993, as well as diltiazem, caused dose-dependent hypotension, a marked increase in vertebral blood flow, an increase in heart rate, and increases in femoral, brachial and common carotid blood flow. Moreover, the peak response of the cardiovascular effect of d-cis-TA-993 was observed immediately after the administration. Thus, the cardiovascular effect of d-cis-TA-993 was very similar to that of diltiazem.

The mechanism of vasodilating action of diltiazem is explained by its blocking action on voltage-dependent L-type calcium channels. In the present study, d-cis-TA-993 also showed vasorelaxation action in the isolated and K⁺-contracted canine femoral artery in the same manner and with similar potency as that of diltiazem. These results suggest the possibility that the cardiovascular action of d-cis-TA-993 is due to a blocking action on voltage-dependent L-type calcium channels such as diltiazem.

l-cis-Diltiazem and TA-993 also showed concentration-dependent vasorelaxation on 40 mM K⁺-induced contractions in the isolated canine sapheous artery. However, the vasorelaxing activities of l-cis-isomers were much weaker than those of d-cis-isomers. In addition, the antagonistic activity of TA-993 against Ca²⁺ in the isolated canine sapheous artery was much weaker than that of diltiazem. Thus, the mechanism of increasing action of these l-cis isomers on limb blood flow cannot be explained by the blocking action on voltage-dependent L-type calcium channels. The increasing action of TA-993 on femoral blood flow was completely inhibited by pretreatment with hexamethonium, a blocker of autonomic ganglia. In addition, pretreatment with neither propranolol nor atropine influenced the increasing action of TA-993 on femoral blood flow (our preliminary study). Therefore, the involvement of an α-adrenergic receptor in the increasing action of TA-993 on limb blood flow is suggested. The mechanism of selective increasing action of l-cis-diltiazem on limb blood flow might be the same as that of TA-993.

In conclusion, our results indicate that TA-993 shares a common profile with l-cis-diltiazem, and suggest that 1,5-benzothiazepine derivatives of a l-cis configuration are a different class of drug from that of the d-cis configuration.

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