STUDIES ON A NEW 1, 5-BENZOTHIAZEPINE DERIVATIVE (CRD-401)
II. VASODILATOR ACTIONS

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In a previous report (1), it was shown that a new 1, 5-benzothiazepine derivative, 3-acetoxy-2, 3-dihydro-5-[2-(diethylamino)ethyl]-2-(p-methoxyphenyl)-1, 5-benzothiazepine-4 (5H)-one hydrochloride, produced a potent coronary vasodilator effect in anesthetized dogs. Among four stereoisomers of the compound, d- and l-isomers of cis- and trans-forms, the d-cis-isomer (CRD-401) exhibited the most powerful effect when injected into the femoral vein. Without increase in myocardial oxygen consumption, a significant increase in coronary blood flow resulted.

The present report deals with the vasodilator action of d-cis-isomer of the new benzothiazepine derivative under the influence of various pharmacological blockers. The results are considered in relation to the mechanism of the compound. Effects of dl- and l-cis-isomers and dl-trans-isomer were also investigated and compared with the action of d-cis-isomer. Experiments were performed on the coronary and femoral arteries in anesthetized dogs and on the coronary vessels in isolated hearts of the guinea pig.

MATERIALS AND METHODS

1. Coronary artery blood flow

Male dogs weighing 15–25 kg were anesthetized using sodium pentobarbital (30 mg/kg, i.v.) and the chest was opened by the removal of a portion of the left fourth rib under positive pressure respiration (room air). According to the method of Eckenhoff et al. (2), the blood from the left carotid artery was led to the anterior descending coronary artery by a short extracorporeal loop. The blood flow was then measured by means of an electromagnetic flowmeter (Nihon Koden, MF-2), which was introduced into the loop. Femoral arterial pressure was obtained and recorded via a pressure transducer as well as the coronary blood flow into a multipurpose polygraph (Nihon Koden, RM-150).

2. Femoral artery blood flow

Male dogs (10–14 kg) were anesthetized using sodium pentobarbital (30 mg/kg, i.v.). A short extracorporeal loop was introduced into the left femoral artery and the blood flow was measured by the same procedure described above.

The test compound, in a volume not exceeding 0.5 ml, was injected into the extracorpo-
real loop inserted in the anterior descending coronary artery or left femoral artery. A blocker such as propranolol, atropine or diphenhydramine was introduced into the femoral vein through the cannula, 3 min before administration of the test compound. In all experiments, a dose of drugs not affecting the systemic blood pressure was used. Blood coagulation was prevented using heparin (500 units/kg, i.v.).

3. Coronary blood flow in isolated hearts

Langendorff’s method was used for testing the effects of drugs on the coronary blood flow of isolated guinea pig heart (about 300 g). The isolated heart was perfused with 2% of defibrinated rabbit blood dissolved in Lock-Ringer solution, which had been saturated with a mixed gas of 95% O₂ and 5% CO₂. Perfusion pressure was kept at about 40 cm H₂O at the level of the heart. To eliminate spontaneous movement of the heart, AC current (50 Herz, 10 V) was applied to the right auricle and a “fibrillated heart” (3) was prepared. In all cases, 0.1 ml of the drug solution was injected into the aortic cannula and the outflow of the perfusate was measured by means of a drop counter. Experiments were carried out at 25°C.

4. Drugs

Drugs used in the present experiments were: papaverine hydrochloride (Iwaki Seiyaku), propranolol (I. C. 1.), atropine sulfate (Tanabe Seiyaku), diphenhydramine hydrochloride (Tanabe Seiyaku), isoproterenol hydrochloride (Boehringer Sohn), dipyridamole (Boehringer Sohn), adenosine (Kokoku Rayon and Pulp). The new 1, 5-benzothiazepine derivative and its stereoisomers were synthesized in our Organic Chemistry Research Laboratory (4–6). Chemical structure is shown in Fig. 1.

![Fig. 1. Chemical structure of the new 1, 5-benzothiazepine derivative.](image)

RESULTS

1. Effect on coronary blood flow

1) Coronary artery blood flow in dogs

When injected into the anterior descending coronary artery, the new 1, 5-benzothiazepine derivative produced a vasodilator effect at a dose which had no influence on systemic blood pressure. Fig. 2 shows the dose-response curves obtained for d-, dl- and l-cis-isomers and dl-trans-isomer of the compound and papaverine. Action patterns of these compounds are also illustrated in the figure.

As shown in Fig. 2, coronary vasodilator actions of d- and dl-cis-isomers were obviously
longer-lasting than those of l-cis- and dl-trans-isomers and papaverine. When estimated by measuring maximum response, potencies of d-, dl- and l-cis-isomers and dl-trans-isomer were 10, 5, 1 and 0.3 times as active as papaverine, respectively.

It can be seen in Fig. 2 that the l-cis-isomer and papaverine showed a similar monophasic pattern. However, at a higher dose, producing a systemic effect, the pattern of action of papaverine became diphasic, while that of l-cis-isomer remained monophasic. An example of the result is shown in Fig. 3. The second component of papaverine action may be ascribed to its positive inotropic effect (7). Since the benzothiazepine derivative has a weak negative inotropic action (1), a diphasic pattern like papaverine might not be observed.
The effect of propranolol on coronary vasodilator actions of the compounds is shown in Fig. 4. Three min after intravenous administration of propranolol (0.5 mg/kg), the compounds were injected into the coronary artery. As indicated in Fig. 4, the action of isoproterenol was conspicuously inhibited by pretreatment with propranolol, whereas those of d- and l-cis-isomers of the benzothiazepine derivative and papaverine were not affected by propranolol.

b) Effects of atropine

Coronary vasodilator action of acetylcholine was completely inhibited after treatment with atropine (1 mg/kg, i.v.). Atropine, however, did not inhibit the effects of d- and l-fomerer, cis-isomers and papaverine (Fig. 5).

c) Effects of diphenhydramine

As shown in Fig. 6, diphenhydramine (5 mg/kg, i.v.) inhibited the vasodilator action of histamine on the coronary artery. On the contrary, no inhibitory effect was observed on the actions of d- and l-cis-isomers and papaverine.

d) Influences of d- and l-cis-isomers on coronary vasodilator action of adenosine

In present experiments, adenosine was injected into anterior descending coronary artery before and during continuous infusion of the test compound into the femoral vein of the anesthetized dog. It has been reported in a previous paper (1) that the d-cis-isomer of the
Fig. 5. Effect of atropine on the coronary vasodilator actions of acetylcholine (ACh), d- and l-cis-isomers of the benzothiazepine derivative and papaverine. Explanations, see Fig. 4.

Fig. 6. Effect of diphenhydramine on the coronary vasodilator action of histamine (Hist), d- and l-cis-isomers of the benzothiazepine derivative and papaverine. Explanations, see Fig. 4.
FIG. 7. Coronary vasodilator action of adenosine before and during continuous infusion of dipyridamole (3 µg/kg/min) or the d- (10 µg/kg/min) and l-cis-isomers (100 µg/kg/min) of the benzothiazepine derivative. Adenosine (30 ng/kg) was injected into the coronary artery in the anesthetized dog. B.P. = blood pressure; C.B.F. = coronary artery blood flow.

1, 5-benzothiazepine derivative did not potentiate the coronary vasodilator action of adenosine, while dipyridamole strongly intensified it (cf. 8–10). As shown in Fig. 7, the vasodilator action of adenosine was not potentiated either by the d-cis-isomer (101 g/kg/min) or by the l-cis-isomer (100 µg/kg/min).

2) Coronary blood flow in isolated hearts

Effects of compounds on the isolated heart coronary blood flow was examined on the Langendorff's preparation of guinea pig (Fig. 8). As in the case of coronary artery in the dog, the coronary vasodilator action of d-cis-isomer was stronger than that of dl- and l-cis-isomers and papaverine. As shown in Fig. 8, vasodilation caused by the l-cis-isomer was followed by a slight vasoconstriction. The dl-trans-isomer produced a vasoconstrictor effect.
FIG. 8. Effect of the benzothiazepine derivatives and papaverine on the coronary blood flow in the isolated heart of the guinea pig. Ten μg/heart of each compound was injected into the aortic cannula. The ordinate is represented by the logarithmic scale.

FIG. 9. Effect of d-, dl and l-cis-isomers and dl-trans-isomers of the benzothiazepine derivative and papaverine on the femoral artery blood flow in the anesthetized dog. Test compounds were injected into the femoral artery. (A) Dose-response curves estimated by measuring the maximum response; (B) dose-response curves estimated by measuring the half-duration time; (C) experimental records. F.B.F. femoral artery blood flow.

2. Effect on femoral artery blood flow

In the foregoing experiments, vasodilator effect on the coronary artery was examined.
To test whether or not a similar effect could be observed in other vascular beds, the action of the femoral artery blood flow was studied in the anesthetized dog.

As shown in Fig. 9, the patterns of test compound actions were quite similar to those observed in the coronary artery: the d- and dl-cis-isomers exhibited longer duration of vasodilator action than the l-cis- and dl-trans-isomers and papaverine. The dose-response curves, which were obtained by measuring the maximum vasodilator response, indicate that the d-cis-isomer was 3 times as active as papaverine, while the dl-cis-isomer had the activity similar to papaverine. On the other hand, the potencies of l-cis- and dl-trans-isomers were about 1/8 and 1/11 of papaverine, respectively.

It is interesting to note that potencies of the benzothiazepine derivatives relative to papaverine were somewhat lower in the femoral than in the coronary artery (cf. Fig. 12).

1) Effect of propranolol

Vasodilator action of isoproterenol on the femoral artery in the anesthetized dog was strongly inhibited by pretreatment with propranolol (0.5 mg/kg, i.v.). On the other hand, no inhibitory effect was observed on the effects of d- and l-cis-isomers and papaverine.

2) Effect of atropine

When injected into the femoral vein, atropine completely inhibited the vasodilator action of acetylcholine, while it had no influence on the effect of the benzothiazepine derivatives and papaverine.

3) Effect of diphenhydramine

Vasodilator effects of d- and l-cis-isomers and papaverine were not inhibited even after treatment with diphenhydramine, which markedly depressed the dilator action of histamine.

DISCUSSION

When administered into the coronary and femoral arteries, the new 1, 5-benzothiazepine derivative, 3-acetoxy-2, 3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1, 5-benzothiazepin-4(5H)-one hydrochloride, produced a vasodilator action on the anesthetized dog. The actions of d- and dl-cis-isomers of the compound were longer-lasting than those of l-cis- and dl-trans-isomers and papaverine.

As was demonstrated in the present experiments, the d-cis-isomer produced the most powerful vasodilator effect on both the coronary and femoral arteries, and the potency was followed by those of dl- and l-cis-isomers and dl-trans-isomer in decreasing order. By intravenous administration. A similar order of potencies was also found on the coronary sinus blood flow in the anesthetized dog (1). Also, as in the case of isolated guinea pig heart coronary vasodilator action of d-cis-isomer was strongest, the dl-cis-isomer the next and the l-cis-isomer the least of the three. The dl-trans-isomer produced vasoconstriction in the isolated heart. From these results, it can be stated that, regardless of the administration route or irrespective of the species of animals or vascular bed, the vasodilator action of the new 1, 5-benzothiazepine derivative was highly stereospecific for the d-cis-isomer.

It has been shown in the foregoing that in activities relative to papaverine, vasodilator effects of the benzothiazepine derivatives on the coronary artery were somewhat stronger
than those on the femoral artery. In addition, it has been demonstrated that the intravenous injection of the d-cis-isomer, which caused about 100% increase in the coronary blood flow, produced only about 25% in the femoral artery blood flow (1). These results suggest that the vasodilator action of the benzothiazepine derivatives are selective for the coronary artery rather than the femoral artery.

Like papaverine, the vasodilator effects of d- and l-cis-isomers of the compound were not inhibited by propranolol, atropine, diphenhydramine, suggesting that the adrenergic, cholinergic or histaminergic mechanism would not be involved in their actions. As demonstrated in the present study, these compounds did not potentiate the coronary vasodilator action of adenosine. It was also found that after intra-arterial administration, all the compounds tested caused a vasodilation on the coronary and femoral arteries at a dose that did not affect systemic blood pressure. Based on these facts, it is assumed that the 1,5-benzothiazepine and its stereoisomers have a property acting directly on the blood vessels.

As reported previously (1), the d-cis-isomer produced an increase of coronary blood flow in dog heart-lung preparation. It also increased coronary blood flow when myocardial oxygen consumption diminished. Furthermore, as will be shown in the succeeding report (11), the d-, dl- and l-cis-isomers of the benzothiazepine derivative produced a relaxing effect on the isolated smooth muscle in a manner similar to papaverine. All these facts support the above assumption about the property of the test compound.

It is worth noting that in spite of having a property which acts directly on blood vessels, the vasodilator action of the new 1,5-benzothiazepine derivative is highly stereospecific for the d-cis-isomer.

**SUMMARY**

The vasodilator effect of the new 1,5-benzothiazepine derivative was studied by intraarterial administration to dogs and isolated guinea pig hearts.

1. Actions of d- and dl-cis-isomers of the compound on the coronary and femoral arteries were longer-lasting than those of l-cis- and dl-trans-isomers in the anesthetized dog. The d-cis-isomer produced the most powerful effect and the potency was followed by those of dl- and l-cis-isomers and dl-trans-isomer in decreasing order. Similar order of potencies was also found in the guinea pig, except for the effect of dl-trans-isomer which resulted in vasoconstriction.

2. Vasodilator effect of d- and l-cis-isomers of the benzothiazepine derivative were not inhibited by propranolol, atropine or diphenhydramine, suggesting that adrenergic, cholinergic or histaminergic mechanisms would not be involved in their actions. These compounds did not potentiate the coronary vasodilator action of adenosine.

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REFERENCES

1) SATO, M., NAGAO, T., YAMAGUCHI, I., NAKAJIMA, H. AND KIYOMOTO, A.: *Arzneim.-Forsch.* 21, 1338 (1971)

2) ECKENHOFF, J.E., HAFFENSEHIEL, J.H. AND LANDMESSER, G.H.: *Am. J. Physiol.* 148, 258 (1947)

3) CHARLIER, R.: *Coronary Vasodilators*, Pergamon Press, Oxford (1961)

4) KUGITA, H., INOUE, H., IKEZAKI, M. AND TAKEO, S.: *Chem. Pharm. Bull.* 18, 2028 (1970)

5) KUGITA, H., INOUE, H., IKEZAKI, M., TAKEO, S. AND KONDA, M.: *Chem. Pharm. Bull.* 18, 2284 (1970)

6) KUGITA, H., INOUE, H., IKEZAKI, M., TAKEO, S. AND KONDA, M.: *Chem. Pharm. Bull.* 19, 595 (1971)

7) DARLY, Y.D., SPROUSE, J.H. AND WALTON, R.P.: *J. Pharmac. exp. Ther.* 122, 386 (1958)

8) BREITSCHEIDER, H.J., FRANK, A., BERNARD, V., KOCCHRIEK, K. AND SCHOLER, F.: *Arzneim.-Forsch.* 9, 49 (1959)

9) STAFFORD, A.: *Br. J. Pharmac. Chemother.* 28, 218 (1966)

10) NOTT, M.W.: *Br. J. Pharmac. Chemother.* 39, 287 (1970)

11) NAGAO, T., SATO, M., IWASAWA, Y., TAKADA, T., ISHIDA, R., NAKAJIMA, H. AND KIYOMOTO, A.: *Jap. J. Pharmac* (in press)