CEREBRAL VASODILATION AND SPASMOLYTIC ACTIVITY OF DILTIAZEM IN ANESTHETIZED ANIMALS

Sakae MURATA, Taku NAGAO and Hiromichi NAKAJIMA
Pharmacological Research Laboratory, Tanabe Seiyaku Co., Ltd., Toda, Saitama 335, Japan
Accepted June 26, 1982

Abstract—The effects of diltiazem on the cerebral blood flow and cerebrovascular spasm were studied in pentobarbital anesthetized animals. In Rhesus monkeys, the common carotid and internal carotid blood flow were measured by an electromagnetic flowmeter. Diltiazem (10–300 µg/kg, i.v.) dose-dependently increased both the common carotid and internal carotid blood flow, and the increase in internal carotid blood flow persisted for a longer period than that in the common carotid blood flow. In dogs, regional blood flow in the cerebral cortex (rCBF) was measured by means of the hydrogen gas clearance method. Diltiazem (20 µg/kg/min, i.v.) increased rCBF by about 20% of the control during the infusion, and the increase in rCBF was still continued 40 min after the infusion was stopped. In cats, the basilar artery was exposed by craniotomy through the transcerico-transclival approach, and vasospasm was induced by topical administration of 5-HT, PGF₂α and incubated blood. Diltiazem, either applied topically to the artery (100 µg/ml) or infused continuously into the femoral vein (20 or 40 µg/kg/min), suppressed the vasoconstriction evoked by the spasmogen.

Diltiazem is a potent coronary vasodilator with calcium antagonistic activity (1–5). Among various vascular beds tested, the vasodilating effect of diltiazem was especially remarkable in the coronary and vertebral arteries (6, 7). On the cerebral circulation, however, detailed experiments have not been carried out yet, except for the finding that diltiazem increased the vertebral blood flow in the anesthetized dog (7). Therefore, we studied the effects of diltiazem on the internal carotid blood flow as well as common carotid blood flow in the monkey, on the cerebral cortical blood flow in the dog, and on the cerebral vasospasm in the cat.

MATERIALS AND METHODS
1. Effects of diltiazem on the common carotid and internal carotid blood flow in anesthetized monkeys: Male Rhesus monkeys weighing 3.9 to 4.9 kg were anesthetized with ketamine, 5 to 10 mg/kg i.m., followed by i.v. administration of 15–20 mg/kg sodium pentobarbital (PB). Thereafter, PB, 3 mg/kg/hr i.v., was infused throughout the experiment to keep anesthesia constant. After the cervical incision, the trachea was intubated, and the animal was artificially ventilated with a respirator under the condition of 20–30 ml/stroke, 40 strokes/min (Harvard, Model-681). The left common carotid blood flow and right internal carotid blood flow were measured by an electromagnetic flowmeter (Nihon Kohden, MF-26, 27). In this case, the right internal carotid blood flow was determined after ligation of
the external carotid artery, lingual artery and superior thyroidal artery. Arterial blood pressure was measured by a pressure transducer (Nihon Kohden, MPU-0.5) connected to the cannulated femoral artery. Heart rate was measured by a cardiotachometer that was triggered by the arterial pulse. Drugs dissolved in saline were administered via polyvinyl tubing inserted into the femoral vein. For measuring partial pressures of gases (Po2, Pco2) and pH, arterial blood was sampled from the brachial artery, and analyzed by a blood gas meter (Radio-Meter, BMS-MK2 and PHM-72).

2. Effect of diltiazem on the cerebral cortical blood flow in anesthetized dogs: Male mongrel dogs weighing 10 to 18 kg were anesthetized with PB, 30 mg/kg i.v.; and 4 to 5 mg/kg/hr of PB was subsequently administered intravenously during the experiment. The trachea was intubated and artificial ventilation was performed (15 ml/kg/stroke, 20 strokes/min, Takashima, Model-100). After the head was fixed with a stereotaxic apparatus, parietal skin and muscle were removed, and the dura mater was reached by craniotomy of 16 to 20 mm diameter. The cerebral cortex was carefully exposed with an incision of the dura mater so as not to injure the cortical tissue.

For the hydrogen gas clearance test, electrodes, which were made from a 0.3 mm thick and 10 mm long platinum wire with a platinum-black plated tip, were inserted into the cortical tissue of the lateral gyrus or entolateral gyrus to a depth of 1 to 2 mm. A plate-type electrode made of silver-silver-chloride was placed subcutaneously as a reference electrode. Hydrogen gas was inhaled for 3 min into the inspiring air at a concentration of 5 to 10% to saturate the tissue. After inhalation was stopped, the desaturation curve of hydrogen pressure in the cortical tissue was monitored by a hydrogen gas clearance flowmeter (Unique-Medical, UH-METER, PHG-201). The procedure for obtaining the blood flow was as follows: Hydrogen clearance curves were plotted on a semilogarithmic scale, half-time ($T_{1/2}$) of hydrogen desaturation was determined, and tissue blood flow was calculated from the formula derived from Aukland et al. (8).

$$\text{Blood flow} = \frac{0.693}{T_{1/2}} \text{ml/min (} \lambda = 1)$$

When the clearance curve was biexponential, $T_{1/2}$ was determined by the initial slope method in which the clearance curve for 4 to 5 min except for the initial 1 min was employed (9).

In several experiments, partial pressures of gases in the arterial blood and right vertebral blood flow were monitored together with the cerebral cortical blood flow; arterial blood was withdrawn from the cannulated femoral artery for measuring Po2 and Pco2 and right vertebral blood flow was measured by an electromagnetic flowmeter. Arterial blood pressure and heart rate were monitored during the experiment. Drugs dissolved in saline were administered into the femoral vein.

3. Effect of diltiazem on the cerebral vasospasm in anesthetized cats: Cats of either sex weighing 1.5 to 4.0 kg were anesthetized with intraperitoneal injection of PB (30 mg/kg) and with subsequent i.v. administration of PB, 4 to 5 mg/kg/hr. After cervical incision, the trachea was intubated, and cats were artificially ventilated (15 ml/kg/stroke, 25 strokes/min, Harvard, Model-681). Animals were fixed in supine position with a stereotaxic apparatus. After putting aside the trachea and esophagus, the longus colli muscle was cut off to expose the clivis. The clivis of 10 mm × 5 mm was removed by a dental drill, and the basilar artery was exposed by a careful incision of dura mater so as not to injure the artery and brain.

Experimental cerebral vasospasm was
evoked as follows: After aspirating off the cerebro-spinal fluid from the subdural cavity, the spasmogen was dropped into the cavity to dip the basilar artery for 5 min. Then the spasmogen was aspirated and was removed by rinsing the subdural cavity with saline more than three times. Twenty-five min after removal of the spasmogen, the preparation was released from the spasm, and the diameter of basilar artery almost completely returned to the initial size. After a constant spasm was obtained by repetitive trials, the anti-spasmogenic effect of diltiazem was examined by topical and intravenous administration. In topical application studies, diltiazem (100 μg/ml) was simultaneously administered to the subdural cavity with the spasmogen for 5 min. In intravenous dosing, infusion of diltiazem (20 or 40 μg/kg/min) was started 15 min before the administration of the spasmogen.

To evaluate the degree of vasospasm, the inner diameter of the basilar artery was measured under the binocular microscope (magnification, ×20), just before and at 1, 2, 3, 4 and 5 min after application of 5-HT or PGF2α or at 2 and 5 min after application of incubated blood. The diameter of the artery was again measured at 5, 15 and 25 min after the spasmogen was washed out.

As spasmogens, we used 5-HT (30 μg/ml in saline solution), PGF2α (30 μg/ml in saline solution), and incubated blood prepared from cats. The incubated blood was prepared as follows: Blood was withdrawn from the femoral artery or vein of cats under light anesthesia by ketamine, and it was incubated for 7 to 10 days at 37°C. Before experiments, the incubated blood was centrifuged for 20 min at 3,000 rpm. The supernatant was used as the spasmogen.

Statistical analysis of the data was made by t-test, and data was considered significant where P<0.05.

RESULTS

1. Effects of diltiazem on the common carotid and internal carotid blood flow in anesthetized monkeys

Figure 1 shows the dose-response curves for the effects of diltiazem on the common carotid blood flow (CCBF), internal carotid blood flow (ICBF), mean arterial blood pressure (MABP) and heart rate (HR). Papaverine was used as the reference compound. As shown in Fig. 1, intravenous injection of diltiazem (10–300 μg/kg) and papaverine (30–300 μg/kg) caused a dose-dependent increase in CCBF; and at doses less than 300 μg/kg, the effect of diltiazem was about three times as potent as that of papaverine. At 300 μg/kg, both compounds exhibited similar potency. Figure 1 also demonstrates that diltiazem and papaverine increased ICBF in a dose-dependent manner, and the activity of diltiazem was three times
more potent than that of papaverine. On the other hand, MABP was reduced by both diltiazem and papaverine; diltiazem produced a more potent effect than papaverine. HR was increased by papaverine, while diltiazem slightly increased HR at lower doses but reduced it at higher doses.

Figure 2 illustrates time-courses of the increase in CCBF and ICBF after intravenous dosing of diltiazem (300 μg/kg) and papaverine (1,000 μg/kg). The results indicated that the duration of action of diltiazem was obviously longer-lasting than that of papaverine and that ICBF increased by diltiazem was maintained for a longer period than CCBF.

In addition to these findings, it was demonstrated that PO₂ (96 mmHg, n=3) and PCO₂ (30 mmHg, n=3) in the arterial blood were not affected by diltiazem (300 μg/kg, i.v.).

2. Effect of diltiazem on the cerebral cortical blood flow in anesthetized dogs

Figure 3 shows the effect of diltiazem on the cerebral cortical blood flow (rCBF) as measured by means of the hydrogen gas clearance method. When diltiazem was administered intravenously at a rate of 20 μg/kg/min for 30 min, rCBF increased by approx. 20% of the control; and its effect persisted at least for approx. 15 min after the

![Fig. 2. Time courses for the increase in the common carotid blood flow (CCBF) and internal carotid blood flow (ICBF) after intravenous administration of diltiazem and papaverine in anesthetized monkeys. Each point represents the mean of six experiments. The point and bar outside the curves are the mean value and standard error (n=6) of maximum responses (ordinate) and of times of maximum responses (abscissa) to the drug: diltiazem (300 μg/kg, i.v., ○), papaverine (1,000 μg/kg, i.v., ●).](image)

![Fig. 3. Effects of diltiazem on the cerebral cortical blood flow (rCBF), vertebral blood flow (VBF), mean arterial blood pressure (MABP) and heart rate (HR) in anesthetized dogs. Diltiazem was administered intravenously at a rate of 20 μg/kg/min for 30 min. Each point and bar represent the mean value and standard error of seven experiments. The interval between each trial of H₂-clearance is about 30 min. *, **significantly different from the trial of C2 at P<0.05 and P<0.01, respectively.](image)

| Table 1. Effects of diltiazem (20 μg/kg/min, i.v. infusion) on PO₂ and PCO₂ in the arterial blood of anesthetized dogs. Each value is the mean±standard error of five experiments |
|-----------------|-----------------|-----------------|
|                  | Before infusion| During infusion  | After infusion |
| PO₂             | 104.3±10.8      | 104.8±9.7       | 106.0±12.1     |
| PCO₂            | 25.7±4.1        | 24.4±3.0        | 25.6±2.7       |
infusion of diltiazem was stopped. Increase in rCBF could still be observed 40 min after cessation of the infusion. On the other hand, the vertebral blood flow (VBF) was raised by approx. 200% of the control during the infusion of diltiazem, and the increased VBF gradually decreased to the control level after the infusion was stopped. MABP was reduced and HR was increased by diltiazem. As summarized in Table 1, during the infusion of diltiazem, no change was discernible in Po2 and Pco2 in the arterial blood.

3. Effect of diltiazem on the cerebral vasospasm in anesthetized cats

3-1. Topical administration of diltiazem:
The topical administration of 5-HT (30 μg/ml), PGF2α (30 μg/ml) or incubated blood to the subdural cavity induced a strong vasoconstriction in the basilar artery. Five min after the spasmogen was removed by washout, the vasoconstriction induced by the spasmogen was gradually reduced; and the initial diameter was regained by 15 or 20 min after the removal (Figs. 4-6).

When applied to the subdural cavity simultaneously with 5-HT or PGF2α, diltiazem (100 μg/ml) completely prevented the spasmogen-induced vasoconstriction; and moreover, it increased the diameter of the basilar artery over the initial size (Figs. 4 and 5). The vasodilation thus elicited was still maintained for 25 min after the removal of diltiazem and the spasmogen. Reintroduction of 5-HT evoked a weak vasoconstriction (Fig. 4), while PGF2α produced vasospasm comparable to the control experiment (Fig. 5). As demonstrated in Fig. 6, the spasmodic effect of incubated blood was also antagonized with diltiazem. On the other hand, when reintroduced into the cavity, the incubated blood caused vasoconstriction to the same extent as the control.

3-2. Intravenous injection of diltiazem:
In the present experiments, diltiazem was continuously infused into the femoral vein.
while a spasmogen such as 5-HT (30 μg/ml), PGF$_{2a}$ (30 μg/ml) or incubated blood was topically applied to the subdural cavity for 5 min. As shown in Fig. 7, intravenous infusion of diltiazem (20 μg/kg/min) suppressed the vasoconstriction induced by 5-HT or PGF$_{2a}$. The effect of diltiazem on the vasoconstriction induced by incubated blood is shown in Fig. 8. Diltiazem (20 and 40 μg/kg/min) not only suppressed the vasoconstriction dose-dependently, but it accelerated the rate of recovery from the spasm following washout of the incubated blood. Thirty min after cessation of the infusion of a higher dose of diltiazem (40 μg/kg/min), the incubated blood alone was again introduced into the cavity. The vasospasm thus evoked was significantly weak, and its effect was much the same as that obtained in the presence of diltiazem. Figure 8 also shows that diltiazem at a higher dose increased the initial diameter of the basilar artery before administration of the spasmogen; the effect of which is ascribed to the vasodilating of diltiazem itself.

**DISCUSSION**

Diltiazem relaxes the vascular smooth muscle (4, 10) and reduces the myocardial contractile force (5) due to its calcium antagonistic property. It has been reported that the vasodilating activity of diltiazem was not the same among various vascular smooth muscles, being more potent in the coronary artery than in the thoracic aorta and femoral artery (10). Such differences in the sensitivity of diltiazem to vascular beds have been also
demonstrated in the anesthetized dog; diltiazem exerted remarkable increases in both the coronary and vertebral blood flow, in comparison with the increases in the common carotid, femoral, superior mesentric and renal blood flows (6, 7). On the other hand, the effects of diltiazem on the cerebral circulation have not yet been examined in detail, except for the study on vertebral blood flow in anesthetized dogs (7). In dogs, however, the increase in vertebral blood flow does not necessarily represent the increase in the cerebral blood flow itself because there are several anastamoses between the extracranial and intracranial circulation (11, 12). Therefore, in the present experiments, the effects of diltiazem on the cerebral cortical blood flow in anesthetized dogs were measured by means of the hydrogen gas clearance method which is very useful for evaluating the parenchymal blood flow of tissues (8, 13, 14). As demonstrated, diltiazem (20 μg/kg/min, i.v.) increased the cerebral cortical blood flow, and its effect was still maintained 40 min after administration of diltiazem was stopped.

Intravenous injection of diltiazem (10–300 μg/kg) also caused a dose-dependent increase in the internal carotid blood flow as well as in the common carotid blood flow in anesthetized Rhesus monkeys. Its activity was three times more potent and was longer-lasting than that of papaverine. In addition, the effect of diltiazem on the internal carotid blood flow continued for a longer period than that on the common carotid blood flow. It has been shown that the vascular construction of the Rhesus monkey whose anastamoses between the extracranial circulation and internal carotid arteries are very little resembles that of humans (15).

On the other hand, the cerebral blood flow is known to be remarkably increased by the elevation of Pco₂ in the blood (16). The present results indicated, however, that the Pco₂ as well as the Po₂ was almost kept constant during the increase in cerebral blood flow caused by diltiazem.

From these lines of evidence, it is suggested that diltiazem actually causes the increase in the parenchymal blood flow in the brain and that its effect is not ascribed to the elevation of Pco₂ in the blood, but due to its direct action on the vascular smooth muscle.

Cerebral vascular spasm occurs from 2 to 3 days to 2 weeks after subarachnoid hemorrhage and is followed by a heavy cerebral ischemia (17). Such a vasospasm is thought to be derived from the spasmogen released into the intracranium. As the candidates of spasmogens, 5-HT, norepinephrine, prostaglandins, especially PGF₂α, oxyhemoglobin and others have been proposed by many investigators (18, 19). Among them, oxyhemoglobin seems to be one of the most important factors for cerebral vasoconstriction (19) at the present time. In the present study, it was clearly demonstrated that diltiazem, when administered topically (100 μg/ml) or intravenously (20 or 40 μg/kg/min), produced an anti-spasmogenic effect on the spasm of the basilar artery induced by 5-HT, PGF₂α or incubated blood in anesthetized cats. The main vasoconstricting substance in the incubated blood has been reported to be probably oxyhemoglobin (19). These findings suggest that diltiazem may improve the cerebral vasospasm after subarachnoid hemorrhage.

It has been reported that Ca-antagonists including diltiazem relaxed Ca²⁺- or PGF₂α-induced contraction of the isolated cerebral artery more strongly than that of the superior mesentric artery (20, 21). Therefore, it is inferred that the anti-spasmogenic effect of diltiazem, as demonstrated in the present experiments, is due to suppression of the acceleration of Ca²⁺-influx caused by the spasmogen.
Acknowledgement: We thank Mr. Takashi Yamaguchi for his technical assistance.

REFERENCES

1) Nagao, T., Sato, M., Nakajima, H. and Kiyomoto, A.: Vasodilator actions of new 1,5-benzothiazepine derivatives and structure-activity relationships—Differences in biological activities among stereoisomers—. In Structure-Activity Relationships in Drug Actions, Edited by Ishida, U. and Muraoka, S., p. 157–173, Hirokawa Publishing Co., Tokyo (1974) (in Japanese)

2) Flechenstein, A., Nakayama, K., Flechenstein-Grün, G. and Byon, Y.K.: Interactions of vasoactive ions and drugs with Cadependent excitation-contraction coupling of vascular smooth muscle. In Calcium Transport in Contraction and Secretion. Edited by Carafoli, E., Clementi, F., Drabikowski, W. and Margreth, A., p. 566, North-Holland Publishing Company, Amsterdam (1975)

3) Nagao, T., Ikeo, T. and Sato, M.: Influence of calcium ions on responses to diltiazem in coronary arteries, Japan. J. Pharmacol. 27, 330–332 (1977)

4) Takenaga, H., Magaribuchi, T. and Nakajima, H.: Effects of diltiazem on guinea pig portal vein in hypertonic solution, Japan. J. Pharmacol. 28, 457–464 (1978)

5) Nakajima, H., Hoshiyama, M., Yamashita, K. and Kiyomoto, A.: Effect of diltiazem on electrical and mechanical activity of isolated cardiac ventricular muscle of guinea pig. Japan. J. Pharmacol. 25, 383–392 (1975)

6) Sato, M., Nagao, T., Yamaguchi, I., Nakajima, H. and Kiyomoto, A.: Pharmacological studies on a new 1,5-benzothiazepine derivative (CRD-401). 1. Cardiovascular action. Arzneimittelforsch 21, 1338–1343 (1971)

7) Nagao, T., Murata, S., Ikezawa, K., Ikeo, T., Naria, H. and Sato, M.: Effects of diltiazem on hemodynamics and His Bundle electromogram in the anesthetized dog. Folia Pharmacol. Japon. 77, 195–203 (1981) (Abs. in English)

8) Aukland, K., Bower, B.F. and Berliner, R.W.: Measurement of local blood flow with hydrogen gas. Circ. Res. 14, 164–187 (1964)

9) Olesen, J., Paulson, O. and Lassen, N.: Regional cerebral blood flow in man determined by the initial slope of the clearance of intra-arterially injected $^{133}$Xe. Stroke 2, 519–540 (1971)

10) Nakajima, H., Nosaka, K. and Hoshiyama, M.: Effects of diltiazem on the positive inotropic and vasoconstrictor responses to ouabain in vitro. Japan. J. Pharmacol. 27, 910–914 (1977)

11) D’Aleyc, L.G. and Feigl, E.O.: Sympathetic control of cerebral blood flow in dogs. Circ. Res. 31, 267–283 (1972)

12) Miller, M.E., Christensen, G.C. and Evans, H.E.: Anatomy of the dog. p. 941, W.B. Saunders Co., Philadelphia (1964)

13) Yong, W.: $H_2$ clearance measurement of blood flow: a review of technique and polarographic principles. Stroke 11, 552–564 (1980)

14) Haining, J.L., Turner, M.D. and Pantall, R.M.: Measurement of local cerebral blood flow in the unanesthetized rat using a hydrogen clearance method. Circ. Res. 23, 313–324 (1968)

15) Handa, J., Meyer, J.S. and Yoshida, K.: Regional pharmacologic responses of the vertebral and internal carotid arteries. J. Pharmacol. Exp. Ther. 152, 251–264 (1965)

16) Olesen, J.: Cerebral blood flow: methods for measurement regulation, effect of drugs and changes in disease. Acta Neurol. Scand., Supp. 57, 11–18 (1974)

17) Zervas, N.T.: Cerebral vasospasm—Introduction —. In Cerebrovascular Diseases "Eleventh Princeton Conference", Edited by Price, T.R. and Nelson, E., p. 269–271, Raven Press, New York (1978)

18) White, R.P.: Multiplex origins of cerebral vasospasm. In Cerebrovascular Disease "Eleventh Princeton Conference", Edited by Price, T.R. and Nelson, E., p. 307–319, Raven Press, New York (1978)

19) Sonobe, M. and Suzuki, J.: Vasospasmogenic substance produced following subarachnoid hemorrhage, and its fate. Acta Neurochir. (Wien) 44, 97–106 (1978)

20) Hayashi, S. and Toda, N.: Inhibition by Cd$^{2+}$, verapamil and papaverine of Ca$^{2+}$ induced contractions in isolated cerebral and peripheral arteries of the dog. Br. J. Pharmacol. 60, 35–43 (1977)

21) Shimizu, K., Ohta, T. and Toda, N.: Evidence for greater susceptibility of isolated dog cerebral arteries to Ca antagonists than peripheral arteries. Stroke 11, 261–266 (1980)