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Short communication

Cilostazol, a selective cAMP phosphodiesterase inhibitor, dilates retinal arterioles and increases retinal and choroidal blood flow in rats

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Abstract

The effects of cilostazol, a selective cyclic AMP (cAMP) phosphodiesterase inhibitor, on retinal and choroidal blood flow and retinal arteriole diameter were examined in anesthetized rats. The retinal and choroidal blood flow was measured using laser Doppler flowmetry, and the diameter of the retinal arterioles was measured using digital video microscopy. Cilostazol was administered by two routes; systemically via the intravenous route, and directly into the retinal vessels via the intra-arterial route. When administered intravenously, 1 mg/kg of cilostazol produced a biphasic blood flow response, composed of an initial decrease which was dependent on a depressor response of mean arterial pressure, and a subsequent slight but significant increase which was independent of changes in mean arterial pressure. When administered intra-arterially over a 2-min period, 40–55 and 400–440 μg of cilostazol both produced an increase in the blood flow in a dose-dependent manner, while a depressor effect was observed only at the dose of 400–440 μg. The diameter of the retinal arterioles was increased after the intra-arterial injection of cilostazol (400 μg). It is concluded that intra-arterially administered cilostazol induces vasodilation of the retinal arterioles of rats, which results in an increase in blood supply to the retina, independent of changes in mean arterial pressure. © 1998 Elsevier Science B.V.

Keywords: Cilostazol; Vasodilation; Blood flow; Retinal arteriole; Laser Doppler flowmetry; Digital video microscopy

1. Introduction

The synthesized antiplatelet agent cilostazol (6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quiolinone, OPC-13013) (Nishi et al., 1983) selectively inhibit platelet cyclic AMP (cAMP) phosphodiesterase (Umekawa et al., 1984). Cilostazol also elevates the cAMP levels of rabbit aorta, resulting in the relaxation of the vascular strips (Tanaka et al., 1988). When administered intravenously to anesthetized dogs, cilostazol increases the blood flow in various arteries, e.g., coronary, internal carotid, vertebral and femoral arteries, and decreases the blood pressure as a result of the reduction of the resistance in the peripheral blood vessels (Shintani et al., 1985). It has been shown that cilostazol, when applied epineurally, increased the blood flow of a sciatic nerve of normal and diabetic rats, suggesting that cilostazol might be used for the treatment of diabetic neuropathy (Kihara et al., 1995). It is of interest to know whether cilostazol can dilate the retinal vessels, leading to an increase of blood supply to the retina, because such an action would be useful for the treatment of diabetic retinopathy. Thus, the first purpose of the present study was to investigate the effects of cilostazol on retinal and choroidal blood flow using laser Doppler flowmetry in anesthetized rats. Cilostazol was administered by two routes; (1) systemically via the intravenous (i.v.) route, and (2) directly to the retinal vessels via the intra-arterial (i.a.) route. We observed an increase in the retinal and choroidal blood flow following the i.a. injection of cilostazol. Secondly, we compared the cilostazol-induced increase in the blood flow with the changes in the diameter of retinal arterioles using digital video microscopy for the measurement of the arteriole diameter.

2. Materials and methods

Eighteen male Wistar rats, 5–13 months old (340–510 g body weight), were used for the experiments. The ani-

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mals were anesthetized with urethane (1.1 g/kg, i.p.). Additional urethane was administered (0.05–0.1 g/kg, i.v.) to maintain the depth of anesthesia, if the systemic blood pressure was observed to fluctuate. Respiration was maintained using an artificial respirator (model 683, Harvard, USA) and end-tidal CO_2, monitored using a gas monitor (1H26, NEC San-ei, Tokyo), was kept at 3–4% by changing the tidal volume and frequency of the respirator. Gallamine triethiodide (10 mg/kg, i.v.) was used for immobilization of the animals during the experiments. The rectal temperature of the rat was maintained at 37 ± 0.5°C using a thermostatically regulated heating pad and lamp (ATB 1100, Nihon Kohden, Tokyo). Mean arterial pressure was recorded continuously through a cannula in a femoral artery. The femoral vein was cannulated for the i.v. administration of drugs.

A cross incision was made in the cornea of one eye. The iris was removed using a coagulator to avoid bleeding. The lens was removed, the vitreous body was carefully removed with forceps, and the retina was exposed taking care to avoid any injury to the retina. In 14 rats, the retinal and choroidal blood flow was measured by a laser Doppler flowmeter (ALF 2100, Advance, Tokyo). The probe of the flowmeter (outer diameter 0.8 mm) was gently placed in contact with the surface of the retina; care was taken to avoid compressing the retina. In four rats, the diameter of retinal arterioles was measured at a magnification of 1000 times by a digital video microscope (VH-6200, Keyence, Osaka) with a high magnification zoom lens (VH-Z 250, Keyence). The rat was positioned on the X–Y table of the lens stand. The image of the retinal microvessels focused on the CCD camera (about 410 000 pixels) was converted into color video signals and the image (at 1-min intervals) was simultaneously stored on a floppy disk for analysis at a later time. The rat retina contains arterioles of 10–100 μm in diameter (Leeson, 1979). Since on many occasions, the vasodilative responses of the smaller vessels have been observed to be greater than those of larger vessels (Adachi et al., 1992; Habazettl et al., 1992), we focused on the response of the smaller arterioles (10–20 μm diameter).

Cilostazol (Otsuka Pharmaceutical, Tokyo), dissolved in 100% dimethylformamide, was administered via two routes: (1) Intravenously through the femoral venous cannula. Cilostazol solution at doses of 0.1 and 1 mg/kg was injected at volumes of 3–5 μl, and then the cannula was flushed with 100 μl of saline. (2) Intra-arterially. The retinal vessels are derived from the ophthalmic artery, which is a branch of the internal carotid artery. Thus, for the injection of cilostazol directly into the retinal vessels, a cannula (outer diameter 0.5 mm, inner diameter 0.2 mm), with the tip pointing upstream, was inserted into the internal carotid artery via the external carotid artery on the side ipsilateral to the operated eye. Cilostazol solution at doses of 2–5, 40–55, 400–440 μg was injected at a volume of 6 μl, and then saline was infused, at a constant speed of 3 μl/min using an infusion pump (CMA/100, Carnegie Medicin, Sweden), to flush the dead space of the cannula. Thus, the duration of the infusion of cilostazol was 2 min. The onset of the injection of cilostazol was determined from the dead volume of the cannula (7–12 μl) and the infusion speed. It was confirmed in advance that the infusion of saline at this speed did not influence the blood flow.

The data obtained are expressed as mean ± S.E.M. The statistical significance was determined by analysis of variance (ANOVA) followed by Dunnet's test.

3. Results

Fig. 1A shows sample recordings of retinal and choroidal blood flow and mean arterial pressure following a bolus i.v. administration of vehicle (4 μl) and cilostazol (1 mg/kg) in a 4 μl volume. The vehicle did not affect either parameter, while 1 mg/kg of cilostazol affected both the blood flow and the arterial pressure. Cilostazol decreased the mean arterial pressure monophasically by about 40 mmHg. The response of blood flow was biphasic; i.e., an initial decrease and a secondary increase. The initial decrease in the blood flow started within 5–10 s following the start of the i.v. injection of cilostazol, reached the minimum value at about 1 min after the injection, and lasted for about 3 min. The secondary increase in the blood

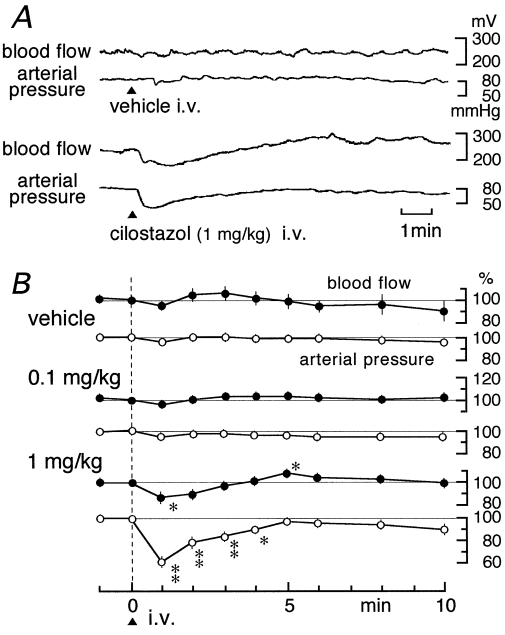

Fig. 1. Effects of the i.v. administration of cilostazol. Sample records (A) and averaged responses (B, in six rats for each cilostazol dose) of retinal and choroidal blood flow and mean arterial pressure. The changes in the blood flow and arterial pressure were calculated every 1 min, and are expressed as percentages of the preinjection values (ordinates). Each point and vertical bar represent a mean ± S.E.M. The onset of the injection is expressed as zero (abscissa). $*P < 0.05$, $**P < 0.01$; significantly different from the preinjection control values.

flow appeared approximately 4 min after the start of the i.v. injection and lasted for several minutes. Fig. 1B summarizes the effects of the vehicle and the two doses of cilostazol (0.1 and 1 mg/kg, i.v.) on the retinal and choroidal blood flow and mean arterial pressure in 6 rats. The 0.1 mg/kg dose had no effect on either parameter. The blood flow and arterial pressure responses induced by 1 mg/kg of cilostazol in 6 rats were essentially the same as those shown in Fig. 1A. The reduced mean arterial pressure reached 62% of the preinjection control level, and then gradually returned to the preinjection level. The blood flow response was biphasic, as described above. The blood flow reached a minimum at 1 min after the start of the injection, falling to 86% of the preinjection control value, and was maximal at 5 min after the start of the injection, reaching 107% of the control.

Fig. 2A shows representative recordings of retinal and choroidal blood flow and mean arterial pressure following the i.a. infusion of vehicle and cilostazol (440 μg) into the internal carotid artery. The vehicle infusion into the same i.a. route had no effect on either parameter, while cilostazol produced a large increase in the blood flow with a slow and slight decrease in arterial pressure. The increase in blood flow started within 30 s, reached the maximum at the end of the infusion, and then gradually returned to the preinjection value within another 5 min. The mean arterial pressure slowly started to decrease about 2 min after the onset of the infusion, and had dropped by about 20 mmHg at approximately one min after the end of the infusion. Fig. 2B summarizes the effect of vehicle and various doses of cilostazol (2–5, 40–55, 400–440 μg, i.a.) on both retinal and choroidal blood flow and mean arterial pressure in five rats. The dose of 2–5 μg cilostazol, as well as the vehicle, were not effective, while the 40–55 μg and 400–440 μg doses of cilostazol increased the blood flow in a dose-dependent manner. The arterial pressure was significantly decreased only at the dose of 400–440 μg. Cilostazol at 40–55 μg produced an increase of the blood flow without any changes in arterial pressure. The dose of 400–440 μg increased the blood flow, but the arterial pressure decreased. The increase of the blood flow following i.a. cilostazol was maximal at the end of the 2-min infusion, reaching 43% (40–55 μg) and 69% (400–440 μg) of the preinjection control levels. The decrease of mean arterial pressure following the infusion of 400–440 μg cilostazol was maximal at 2 min after the end of the infusion, reaching about 15% of the preinjection control level.

To examine the vasodilation effect of cilostazol, we measured the diameter of the retinal arterioles using digital video microscopy. Fig. 2C shows representative photographs of a retinal arteriole under control conditions before the infusion of cilostazol and at 1 min after the end of the infusion of cilostazol (400 μg, i.a.) for 2 min. The retinal arteriole showed an obvious increase in diameter during and following the i.a. infusion of cilostazol. Fig. 2D plots the mean diameter of four retinal arterioles measured

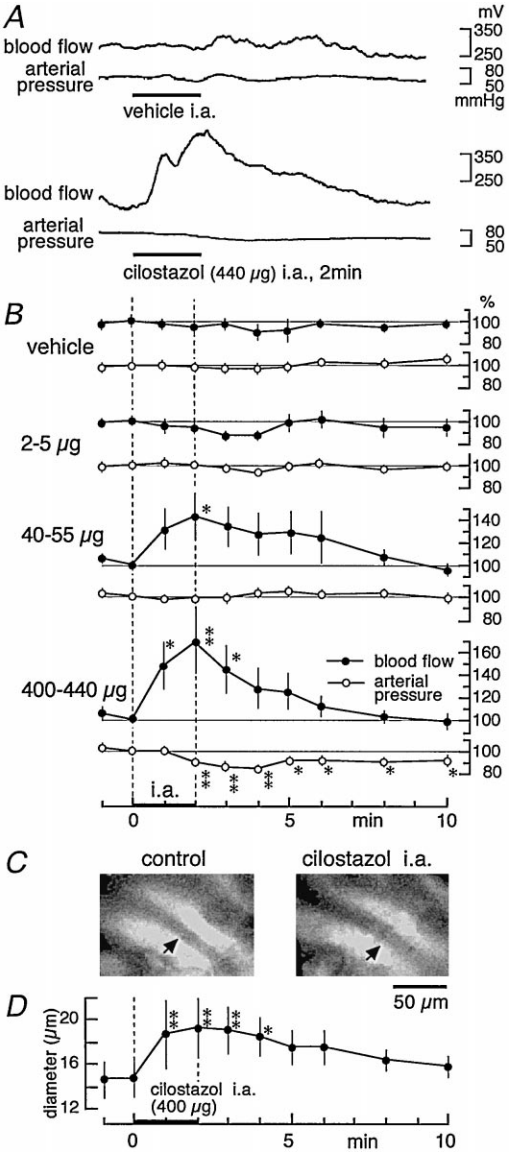

Fig. 2. Effects of the i.a. administration of cilostazol for 2 min. Representative records (A) and averaged responses (B, in five rats for each dose) of retinal and choroidal blood flow and mean arterial pressure. The dashed lines and the heavy bar on the abscissa indicate the time during which the cilostazol was infused. Sample photographs of a retinal arteriole (C) and mean of changes in the diameters of retinal arterioles (D, four rats). See Fig. 1 for other details.

every 1 min in four rats. The mean of diameter of the retinal arterioles measured before the cilostazol infusion was 14.7 ± 1.7 μm, whereas it reached a peak diameter of 19.3 ± 2.7 μm at the end of the infusion.

4. Discussion

The initial decrease in retinal and choroidal blood flow induced by the i.v. injection of cilostazol appeared to be a secondary and passive response caused by the primary depressor response of mean arterial pressure. The depres-

sor response appears to be elicited by vasodilation in various vessels in the heart, hindlimb, etc. (Shintani et al., 1985). The cilostazol-induced secondary increase of the blood flow may thus be an active vasodilative response of retinal and/or choroidal blood vessels elicited by the direct or primary effect of cilostazol on these vessels per se. To prevent the effect of decreased arterial pressure on blood flow, we administered cilostazol intra-arterially into the internal carotid artery and measured the blood flow response with systemic arterial pressure stabilized as much as possible.

In the rats used for the present study, 400–440 μg of cilostazol was equivalent to 1 mg/kg, because the body weight of the rats used was 340–510 g. We speculated that administrations of 400–440 μg i.a. and 1 mg/kg i.v. cilostazol might produce depressor responses of the same magnitude, but the degree and time courses of the responses following the two administrative methods were different. The depressor response following the 1 mg/kg i.v. administration was much greater. The difference might be due to the difference in the two injection routes and speeds.

The increase in retinal and choroidal blood flow following the i.a. infusion of cilostazol into the internal carotid artery appears to be due to the primary vasodilative effect of cilostazol on the retinal and/or choroidal vessels. When cilostazol, at a dose of 400–440 μg, was infused i.a., there was an increase in the blood flow irrespective of any decrease in mean arterial pressure. This pattern following the i.a. injection was different from the pattern that was observed when cilostazol was injected i.v. This difference can be explained as follows. Since the cerebral blood flow occupies 14% of the total blood flow of the body (Bard, 1961) and since the brain is supplied by two internal carotid arteries and two vertebral arteries, the blood flow of one internal carotid artery can be roughly calculated to be 3.5% or slightly more of the total blood flow, indicating that an i.a. administration would bring an approximately 30-times higher concentration of cilostazol to the eye than would an i.v. administration. Thus, the vasodilation effect on retinal and/or choroidal vessels induced by the i.a cilostazol is strong enough to mask the influence of the arterial pressure reduction.

The present study clarified, using video microscopy, that the i.a. infusion of cilostazol into the internal carotid artery actually has a vasodilation effect on retinal arterioles, which is considered to result in an increase in retinal blood flow. An intra-arterial injection can bring effective concentrations of cilostazol to the retinal vessels without systemic effects. Although the blood–retinal barrier will prevent most drugs from reaching the smooth muscle of the retinal vessels (Alm, 1992), cilostazol, having the advantage of being a lipid-soluble compound, can affect the retinal microcirculation, as Kulkarni et al. (1994) showed with the lipid-soluble compound, prostaglandin $F_{2\alpha}$, which constricted retinal vessels in perfused bovine retinal microcirculation preparations.

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