Inhibitory Effect of OP-41483· α -CD, a Prostacyclin Analog, on Peripheral Vascular Lesion Models in Rats

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ABSTRACT—The effect of a chemically stable prostacyclin analog, OP-41483 α -cyclodextrin clathrate (OP-41483· α -CD), on vascular lesions, platelet aggregation and blood pressure were examined and compared with those of prostaglandin E₁ α -cyclodextrin clathrate (PGE₁·CD) in *in vivo* rat models. 1) In the laurate (1 mg/leg, *i.a.*)-induced arterial thrombotic model, OP-41483· α -CD (1 μ g/kg/min, *i.v.*) prevented the progression of femoral arterial vascular lesions and enhanced the development of collaterals in the femoral artery. PGE₁·CD did not inhibit the progression of vascular damages. 2) In the model of vasoconstriction induced by epinephrine (0.05 mg/tail, *s.c.*) and ergotamine (2 mg/kg, *s.c.*), OP-41483· α -CD and PGE₁·CD, at 1 μ g/kg/min, inhibited the progress of tail gangrene and lessened the decrease in tail cutaneous blood flow. 3) OP-41483· α -CD (1 μ g/kg/min) suppressed the ADP (0.1 mg/kg/min, *i.v.*)-induced decrease in the number of circulating platelets without affecting the change in blood pressure. In contrast, PGE₁·CD (3 μ g/kg/min) inhibited ADP-induced thrombocytopenia with a decrease in blood pressure. These results indicate that OP-41483· α -CD has antiplatelet and cutaneous blood flow improving activities that are greater than its hypotensive effect and may be of therapeutic potential in peripheral vascular diseases.

Keywords: OP-41483, Prostacyclin analog, Peripheral vascular lesion, Antiplatelet activity, Prostaglandin E₁.

Prostacyclin is a chemically unstable compound that possesses both potent antiplatelet (1) and hypotensive (2) actions. Because of its clinical potential, various prostacyclin derivatives have been developed with the aims of increasing its chemical stability and dissociating these two actions. OP-41483· α -CD, 5(*E*)-6,9 α -methylene-15-cyclopentyl-16,17,18,19,20-pentanor-PGI₂- α -cyclodextrin clathrate, is a novel and chemically stable prostacyclin analog that inhibits platelet functions (adhesion and aggregation) and prevents thrombus formation in an electrically induced thrombus model in guinea pigs (3). The inhibitory effects of OP-41483· α -CD on platelet functions are produced through dual mechanisms: one mediated by activation of adenylate cyclase and the other by inhibition of Ca²⁺ influx (4). This compound also has a more potent antiplatelet effect than hypotensive activity in baboons (5) and humans (6). Therefore, OP-41483· α -CD may have anti-thrombotic properties for the treatment of thrombotic and ischemic vascular diseases.

In this study, we compared the inhibitory effect of OP-41483· α -CD with that of prostaglandin E₁ α -cyclodextrin clathrate (PGE₁·CD) on two peripheral vascular lesion models in rats: the arterial thrombotic model induced by intraarterial injection of laurate and the vasoconstriction model induced by subcutaneous injection of epinephrine and ergotamine. Furthermore, the effects of both compounds on platelet aggregation (*in vivo*) and blood pressure were examined in conscious rats.

MATERIALS AND METHODS

Animals

Male Wistar rats were purchased from Kitayama Labes and Shizuoka Laboratory Animal Center. All animals were kept in an animal room maintained at 24 $\pm$ 1°C with 60 $\pm$ 10% humidity and illuminated for 12 hr (8:00 AM–8:00 PM). They were fed a standard laboratory diet (Funabashi, MM-5) and tap water *ad libitum*.

Chemicals

OP-41483- α -CD and PGE₁-CD were synthesized at the Ono Research Laboratory. Both compounds were dissolved in physiological saline, and the dose was expressed as the amount of OP-41483 or PGE₁ content. Other drugs used for this study were pentobarbital sodium (Abbott Laboratories, USA), epinephrine (Bosmin[®], Daiichi Pharmaceutical Co.), ergotamine tartrate (Tokyo Kasei Co.) and laurate (Tokyo Kasei Co.). Laurate was dissolved in saline with an equimolar amount of NaOH at a concentration of 10 mg/ml.

Methods

Laurate-induced vascular lesion model: Experiments were carried out according to the methods of Ashida et al. (7). Male Wistar rats weighing 310–449 g were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The right femoral artery was exposed by surgical incision. Vascular lesion was induced by the single injection of laurate (1 mg/leg) into the right femoral artery. The animals were observed for progression of the lesions every day for 9 days. The degree of lesions was rated by the following 5 grades: Grade 1, the region was limited to the nail; Grade 2, the region was limited to the fingers; Grade 3, the region was limited to the half paw; Grade 4, the region was limited to the whole paw; Grade 5, the region was extended to the lower leg.

Catheters connected to rodent swivels (Alice King Chatham Medical Arts) were implanted into the jugular vein under pentobarbital anesthesia (40 mg/kg, i.p.) for the administration of compounds. Saline (0.44 ml/hr) was continuously infused by a pump (Truth, B-II) to conscious rats to prevent blood coagulation in the catheter. OP-41483- α -CD and PGE₁-CD (0.3, 1 and 3 μ g/kg/min) were administered instead of saline from 30 min before the laurate injection for 2 hr and once a day (2 hr) for 9 consecutive days (in conscious rats). On day 9, the left renal artery was cannulated for angiography under pentobarbital anesthesia (40 mg/kg, i.p.). Angiograms were taken [Toshiba Medical; KXO-12(M)] with urografin[®] (Schering Ag.).

Epinephrine and ergotamine-induced tail gangrene model: Experiments were performed by a modification of the method of Lund (8). Male Wistar rats weighing 277–353 g were used. Epinephrine (0.05 mg) was injected subcutaneously at both the ventral and dorsal side at a 6-cm distance from the tip of the rat tail. Simultaneously, ergotamine (2 mg/kg) was injected subcutaneously into the back. The length of gangrene in the tail was measured for 7 days. OP-41483- α -CD and PGE₁-CD (0.1, 0.3 and 1 μ g/kg/min) were infused as described above from 30 min before epinephrine and

ergotamine injections for 7 days (24 hr/day). Cutaneous blood flow of the tail (3-cm length from the tip) was measured under pentobarbital anesthesia (30 mg/kg, i.p.) by a laser Doppler flow meter (Advance, ALF-2000) before and 5 days after the insults.

ADP-induced thrombocytopenia and blood pressure in conscious rats: Male Wistar rats (204–408 g) were used in this study. Cannulas were implanted into the common carotid artery and jugular vein under ether anesthesia. Experiments were started after a few hours recovery. Thrombocytopenia was induced by the intravenous infusion of ADP (0.1 mg/kg/min). The degree of thrombocytopenia was estimated by counting the number of circulating platelets. Blood (20 drops) was withdrawn from the catheter inserted to the common carotid artery into a sample bottle (Onoder-co) 1 min after the start of ADP infusion. The number of platelets was measured by a platelet counter (Toa, PL-0.1). Test compounds were infused intravenously from 30 min before ADP injection until blood sampling. The decrease in platelet number in the test compound-treated animals was compared with that in the saline-treated ones and expressed as percent inhibition. For the measurement of blood pressure, the cannula implanted into the common carotid artery was connected to a pressure transducer (Nihon Kohden, TP-300T) equipped with a carrier amplifier (Nihon Kohden, AP-621G). Systemic blood pressure was recorded on a recorder (Nihon Kohden, RJG-4128). OP-41483- α -CD and PGE₁-CD were infused intravenously for 30 min.

Statistical analyses

Results were expressed as the mean $\pm$ S.E.M. The significance of the data was evaluated using the Mann-Whitney *U*-test or one way ANOVA followed by Dunnett's test. A significance level of more than 95% was taken to be statistically significant ($P < 0.05$).

RESULTS

Effect on the laurate-induced vascular lesion model

Intraarterial injection of laurate (1 mg/leg) caused the progression of femoral arterial occlusive lesions. The paw started to be necrotic 3 days after the injection and then was mummified. Nine days after the insults, 9 out of 11 control animals exhibited lesion of the whole paw (grade 4) or the lesion extended into the lower leg (grade 5). The lesion in the others was limited to half the paw (grade 3), but there was no rat with grades of 1 or 2. The mean intensity of symptoms on day 9 was 4.5 ± 0.3 (Figs. 1 and 3). OP-41483- α -CD (1 μ g/kg/min) significantly inhibited the progression of lesions ($P < 0.01$, Dunnett's test). The lesions in 3 out of 10 rats

Fig. 1. Effect of OP-41483- α -CD on laurate (1 mg/leg, i.a.)-induced peripheral vascular lesions in rats. OP-41483- α -CD was administered intravenously from 30 min before the laurate injection for 2 hr and once a day on consecutive days. Control (●); OP-41483- α -CD: 0.3 μ g/kg/min, i.v. (○); 1 μ g/kg/min, i.v. (△); 3 μ g/kg/min, i.v. (□). Values represent the mean $\pm$ S.E.M. for 10–11 rats. ** $P < 0.01$, compared with the control values (Dunnett's test).

Fig. 2. Effect of PGE₁-CD on laurate (1 mg/leg, i.a.)-induced peripheral vascular lesions in rats. PGE₁-CD was administered intravenously from 30 min before the laurate injection for 2 hr and once a day on consecutive days. Control (●); PGE₁-CD: 0.3 μ g/kg/min, i.v. (○); 1 μ g/kg/min, i.v. (△); 3 μ g/kg/min, i.v. (□). Values represent the mean $\pm$ S.E.M. 10–11 rats.

Fig. 3. Effect of OP-41483- α -CD and PGE₁-CD on laurate (1 mg/leg, i.a.)-induced peripheral vascular lesions in rats (day 9). OP-41483- α -CD and PGE₁-CD were administered intravenously from 30 min before the laurate injection for 2 hr and once a day on consecutive days. * $P < 0.05$, compared with the control values (Mann-Whitney *U*-test).

were limited to the nail (grade 1), and the mean value of the grade on day 9 was 3.0 ± 0.5 . This compound was not effective at 0.3 and 3 μ g/kg/min. PGE₁-CD (1 μ g/kg/min) tended to diminish the development of vascular damages (Figs. 2 and 3). The mean value of the grade on day 9 was 3.5 ± 0.5 , but there was no significant difference from the control.

In the angiographic study, the right femoral artery was completely obstructed at the peripheral site of laurate injection in the control. OP-41483- α -CD, at 1

μ g/kg/min, increased the number of collaterals of the femoral artery compared to that of control rats (Fig. 4). No ameliorative effect was observed in the PGE₁-CD treated groups.

Effect on epinephrine and ergotamine-induced tail gangrene model

Subcutaneous injection of epinephrine and ergotamine caused cyanosis, necrosis and mummification of the tail and finally, the tail fell off. The length of the

Fig. 4. Angiography of the femoral artery in the laurate (1 mg/leg, i.a.)-induced peripheral vascular lesion rats (day 9). Control (A); OP-41483- α -CD, 1 μ g/kg/min, i.v. (B). Arrows indicate the site of laurate injection.

damaged tail in the control was 9.7 ± 0.3 cm 7 days after ischemic insult. OP-41483- α -CD and PGE₁-CD significantly inhibited the development of gangrene at 1 μ g/kg/min (Table 1). Tail cutaneous blood flow in the control was reduced remarkably 5 days after the insults. Both compounds, at 1 μ g/kg/min, lessened the decrease in cutaneous blood flow of the damaged tail compared to that of the control (Table 2).

Effect on thrombocytopenia and blood pressure

Intravenous infusion of ADP (0.1 mg/kg/min) decreased the circulating platelet number by $49.7 \pm 2.2\%$. OP-41483- α -CD (0.3–3 μ g/kg/min) inhibited ADP-induced thrombocytopenia in a dose-dependent fashion (Fig. 5). This compound had antiplatelet activity at 1 μ g/kg/min without causing any blood pressure

changes. On the other hand, PGE₁-CD showed antiplatelet activity at 3 μ g/kg/min and decreased blood pressure at the same dose.

DISCUSSION

Inflammation of blood vessels, thrombus or vasoconstriction may be involved in peripheral vascular diseases (9, 10). Based on these factors, various experimental models in animals have been reported (7, 8, 11). In this study, we examined the effect of OP-41483- α -CD and PGE₁-CD on experimental models of peripheral vascular lesion that were induced by the injection of laurate into the femoral artery (arterial thrombotic model) or by the subcutaneous injection of epinephrine to the tail and subcutaneous injection of ergotamine to the back

Table 1. Effect of OP-41483- α -CD and PGE₁-CD on the length of tail gangrene induced by epinephrine and ergotamine in rats

Compound	Dose (μ g/kg/min)	No. of rats	Length of gangrene (cm)
Control	—	9	9.7 ± 0.3
OP-41483- α -CD	0.1	6	8.8 ± 0.4
	0.3	9	8.5 ± 0.5
	1	8	$7.9 \pm 0.3^*$
PGE ₁ -CD	0.1	8	9.5 ± 0.5
	0.3	7	8.8 ± 0.5
	1	10	$8.1 \pm 0.5^*$

Each value represents the mean $\pm$ S.E.M. * $P < 0.05$, statistical difference from the control (Dunnett's test). Tail gangrene was induced by the subcutaneous injection of epinephrine and ergotamine. The length of tail gangrene was measured 7 days after the insult. OP-41483- α -CD and PGE₁-CD were infused intravenously from 30 min before epinephrine and ergotamine injections for 7 days.

Table 2. Effect of OP-41483- α -CD and PGE₁-CD on tail cutaneous blood flow of epinephrine and ergotamine-induced tail gangrene rats

Compound	Dose (μ g/kg/min)	No. of rats	Tail cutaneous blood flow (ml/min/100 g)	
			pre	day 5
Control	—	9	6.18 ± 0.53	0.82 ± 0.08
OP-41483- α -CD	0.3	9	5.77 ± 0.44	0.98 ± 0.08
	1	9	6.15 ± 0.35	$1.59 \pm 0.13^{**}$
PGE ₁ -CD	0.3	8	6.23 ± 0.34	1.06 ± 0.13
	1	7	5.99 ± 0.33	$1.48 \pm 0.07^{**}$

Each value represents the mean $\pm$ S.E.M. ** $P < 0.01$, statistical difference from the control (Dunnett's test). Tail gangrene was induced by the subcutaneous injection of epinephrine and ergotamine. Cutaneous blood flow of the tail (3-cm length from the tip) was measured by a laser Doppler flow meter. OP-41483- α -CD and PGE₁-CD were infused intravenously from 30 min before epinephrine and ergotamine injections for 7 days.

Fig. 5. Effect of OP-41483- α -CD and PGE₁-CD on thrombocytopenia and blood pressure in conscious rats. Thrombocytopenia was induced by ADP (0.1 mg/kg/min, i.v.). The test compound was infused intravenously from 30 min before the ADP injection. Each point represents the mean $\pm$ S.E.M. for 4–8 rats.

(vasoconstriction model).

The present study demonstrated that OP-41483- α -CD (1 μ g/kg/min) significantly inhibited the progression of femoral arterial vascular lesions induced by laurate with the development of collateral arteries in the femoral artery. Furthermore, in the epinephrine and ergotamine-induced model, OP-41483- α -CD, at 1 μ g/kg/min, attenuated the length of the tail gangrene and lessened the decrease in tail cutaneous blood flow. To test the degree of dissociation between the antiplatelet and hypotensive actions, we examined the effect of this compound on platelet aggregation (in vivo) and blood pressure in conscious rats. Antiplatelet activity in vivo was estimated as the effect of the drug on thrombocytopenia induced by intravenous infusion of ADP. OP-41483- α -CD (1 and 3 μ g/kg/min) inhibited ADP-induced thrombocytopenia in a dose-dependent manner. No hypotensive effect was observed at 1 μ g/kg/min, indicating that OP-41483- α -CD has an antiplatelet activity rather than a hypotensive effect. These observations are consistent with the results obtained in other experiments (3, 5, 6). PGE₁-CD (1 μ g/kg/min) tended to inhibit the progression of laurate-induced vascular damages and significantly inhibited the tail gangrene with an improvement in the decrease in tail cutaneous blood flow. This compound attenuated thrombocytopenia at 3 μ g/kg/min and decreased blood pressure at 1 μ g/kg/min, indicating that PGE₁-CD inhibits platelet aggregation with hypotension in in vivo systems.

Regarding the laurate-induced model, it has been reported that platelet aggregation is a major contributing

factor, in the initiation and the progression of vascular lesions, based on the fact that vascular lesions are suppressed by the treatment with antiplatelet agents or antiplatelet serum (7). Laurate-induced damage of the vascular wall triggers platelet adhesion and aggregation to form occlusive thrombi with some participation of the coagulation system. Therefore, the protective effect of OP-41483- α -CD and PGE₁-CD on laurate-induced vascular lesion must be due to the antiplatelet activity of each compound. The inhibitory effect of OP-41483- α -CD and PGE₁-CD on this model was diminished at 3 μ g/kg/min. This observation might result from the decrease in perfusion pressure induced by hypotension, because the decrease in perfusion pressure could disturb the distribution of the peripheral circulation (12).

In a previous study using the epinephrine and ergotamine-induced model, we observed edema and disorder of the lamina elastica of the blood vessel wall caused by strong vasoconstriction in the early stage, and degeneration of the vascular smooth muscle and thrombosis were detected 24 hr after the insult (13). It has been reported that vasodilators (nitroglycerin or a PGE₁ analog) prevent the tail gangrene, but an anticoagulator (heparin) or antiplatelet agent (ticlopidine) is not effective (8, 13). Therefore, continuous vasoconstriction of the tail may precede thrombosis formation in this model. OP-41483- α -CD and PGE₁-CD, at 1 μ g/kg/min, ameliorated the impaired peripheral circulation induced by vasoconstrictors. These effects of OP-41483- α -CD and PGE₁-CD might be due to the vasodilatory and antiplatelet activities of each compound.

Clinically, vasodilators, antiplatelet drugs, anticoagulants and fibrinolysis activators are employed in the treatment of ischemic ulcer of peripheral vascular diseases. Compounds with multiple actions such as vasodilation and antiplatelet activity are needed, and PGE₁-CD, which has both vasodilating and antiplatelet actions, has been used. In comparison to the activities of PGE₁-CD, OP-41483- α -CD has a more potent antiplatelet activity and an equipotent activity for improving cutaneous blood flow. However, the hypotensive activity of OP-41483- α -CD was less than that of PGE₁-CD, indicating that OP-41483- α -CD has both antiplatelet and peripheral circulation improving activities but does not cause a decrease in blood pressure. Thus, OP-41483- α -CD may be a drug with high therapeutic value for peripheral vascular disease.

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