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Antiplatelet effect of Z-335, a new orally active and long-lasting thromboxane receptor antagonist

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Abstract

We investigated the pharmacological characteristics of Z-335 ((±)-sodium[2-[4-(chlorophenylsulfonylaminomethyl)indan-5-yl]acetate monohydrate), a new indan derivative. Z-335 inhibited the specific binding of [^{3}H]SQ-29548 to human platelets and guinea pig platelet membranes. The IC_{50} values of Z-335 for human platelets and guinea pig platelet membranes were 29.9 ± 3.1 nM with a slope of 1.09 ± 0.05 and 32.5 ± 1.7 nM with a slope of 1.07 ± 0.02, respectively. Z-335 inhibited thromboxane A_2 receptor-mediated human and guinea pig platelet aggregation in vitro and oral administration of this drug to guinea pigs inhibited U-46619- and collagen-induced platelet aggregation for 24 h. Z-335 dose-dependently prevented the occurrence of U-46619-induced pulmonary thromboembolism in mice and the protective effect of this drug (0.3 and 3 mg/kg, p.o.) lasted for 24 h. These results strongly suggest that Z-335 is a potent, orally active and long-lasting thromboxane A_2 receptor antagonist, which may be useful as an antiplatelet drug.

Keywords: Z-335; Indan derivative; Thromboxane A_2 receptor antagonist; Platelet aggregation; Pulmonary thromboembolism

1. Introduction

Thromboxane A_2 released from activated platelets induces platelet aggregation and vasoconstriction (Ellis et al., 1976; Meyers et al., 1979; Smith et al., 1980), which is involved in occlusive arterial thrombosis and vasospasm (Lewis and Smith, 1984; Fitzgerald et al., 1986; Golino et al., 1989; Hirata et al., 1994). Recently, thromboxane A_2 has been reported to stimulate the hypertrophy and proliferation of vascular smooth muscle cells (Ali et al., 1993; Sachinidis et al., 1995) and to activate the adhesion receptors of neutrophils and endothelial cells (Goldman et al., 1991; Ishizuka et al., 1994). Thromboxane A_2 acts through a specific thromboxane A_2 receptor-mediated process (Ushikubi et al., 1989; Hirata et al., 1991, 1996).

Although several selective thromboxane A_2 synthetase inhibitors have been developed, thromboxane A_2 synthetase inhibition by them causes the accumulation of prostaglandin endoperoxide which interacts with thromboxane A_2 receptors to induce platelet aggregation (Hornby and Skidmore, 1982). On the other hand, thromboxane A_2 receptor antagonists, which block the actions of both thromboxane A_2 and prostaglandin endoperoxide, have been reported to show beneficial antiplatelet effects in arterial and venous thrombosis models (Schumacher et al., 1993) and, in part, to prevent the occurrence of myocardial infarction in a clinical study (Savage et al., 1995). Therefore, thromboxane A_2 receptor antagonists may be more beneficial than thromboxane A_2 synthetase inhibitors against occlusive vascular thrombosis associated with platelet activation and vasoconstriction. In particular, a potent, orally active and long-lasting thromboxane A_2 receptor antagonist is expected to ameliorate occlusive vascular thrombosis and to be effective in the treatment of patients with this disease. On the basis of this information, we have recently developed a new indan derivative, Z-335 ((±)-sodium[2-[4-(chlorophenylsulfonylaminomethyl)indan-5-yl]acetate monohydrate). In this study, we investigated the pharmacological characteristics of Z-335 (see Fig. 1 for chemical structure) on platelet thromboxane A_2 receptor binding, platelet aggregation and in a pulmonary thromboembolism model. Vapiprost is a thromboxane A_2 receptor antagonist which is being studied extensively and

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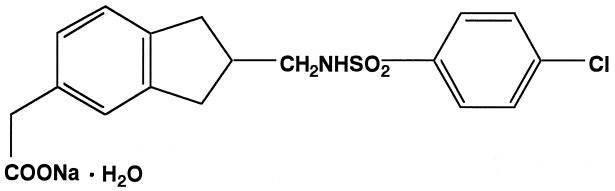

Fig. 1. Chemical structure of Z-335.

daltroban is a lead compound that was used to develop Z-335. Cilostazol has antiplatelet and vasodilator actions, and is a popular antiplatelet drug in Japan. Therefore, we compared the antiplatelet effect of Z-335 with those of vapiprost, daltroban and cilostazol, and clarified the therapeutic utility of this drug as a potent antiplatelet drug.

2. Materials and methods

2.1. Subjects and animals

Blood samples were collected from seven healthy male subjects who had been drug-free for at least 2 weeks. Male Hartley strain guinea pigs (3 weeks old, Nihon SLC, Japan) and male ICR strain mice (4 weeks old, Charles River, Japan) were used in this study. These animals were housed in a temperature-controlled room for at least 5 days before we began the present experiments, which were approved by the Use Committee for Human Samples and Laboratory Animals of the Central Research Laboratories of Zeria Pharmaceutical.

2.2. Materials

Z-335, vapiprost (thromboxane A_2 receptor antagonist), daltroban (thromboxane A_2 receptor antagonist) and cilostazol (type III phosphodiesterase inhibitor) were synthesized in the central research laboratories of Zeria Pharmaceutical. [^{3}H]SQ-29548 (1702.0 GBq/mmol) was purchased from New England Nuclear (USA). U-46619 (thromboxane A_2 receptor agonist, Funakoshi, Japan), arachidonic acid (Sigma, USA), collagen (Collagenreagent Horm, Nycomed, Germany) and ADP (Sigma) were used in this study. Z-335, vapiprost, daltroban and cilostazol were dissolved in dimethylsulfoxide or dimethylsulfoxide–ethanol (1:1) mixture in the in vitro study. In the ex vivo and in vivo studies, these test drugs were suspended in 0.5% (w/v) methyl cellulose solution for oral administration. U-46619 was dissolved in 99.5% (v/v) ethanol and diluted for use with physiological saline. Arachidonic acid and ADP were dissolved in physiological saline.

2.3. Receptor binding studies

Human blood was drawn into a syringe containing a trisodium citrate (0.34%, w/v)–dextrose (0.5%, w/v) mixture and guinea pig blood was collected into a syringe containing trisodium citrate (0.38%, w/v). Platelet-rich plasma was prepared from the human and guinea pig blood by centrifugation at $160 \times g$ for 10 min at room temperature. Human platelet-rich plasma was washed with 12 mM Tris–HCl buffer (pH 7.4) containing 139 mM NaCl and 1.5 mM EDTA and resuspended in 50 mM Tris–HCl buffer (pH 7.4) containing 154 mM NaCl and 10 μM indomethacin to a final density of 300 000 platelets/mm^3. Guinea pig platelet-rich plasma was washed with 50 mM Tris–HCl buffer (pH 7.4) containing 154 mM NaCl, 5 mM EDTA and 10 μM indomethacin and then resuspended with 5 mM Tris–HCl buffer (pH 7.4) containing 5 mM EDTA and 10 μM indomethacin. Finally, the platelet membranes obtained were resuspended in 50 mM Tris–HCl buffer (pH = 7.4) containing 5 mM EDTA and 10 μM indomethacin. The binding assay for human platelets and guinea pig platelet membranes was performed by incubating the platelet samples with 5 nM [^{3}H]SQ-29548 in a total volume of 250 μl at 25°C for 30 min (human platelets) or 20 min (guinea pig platelet membranes). After the incubation, ice-cold physiological saline was added to the samples and the binding reactions were immediately terminated by rapid filtration (Brandel MT-24 cell harvester). Then, the glass-fiber filters were washed twice (human samples) or three times (guinea pig samples) with ice-cold physiological saline. The radioactivity on the filter was counted in a liquid scintillation counter (Packard, TRI-CARB4640) and the IC_{50} values (50% inhibiting concentration) and slope factors were calculated by pseudo-Hill analysis.

2.4. Platelet aggregation in vitro

Human blood from normal volunteers was drawn into a syringe containing trisodium citrate (0.38%, w/v) via venipuncture. Guinea pig blood was collected under ether anesthesia from the abdominal aorta into a syringe containing trisodium citrate (0.38%, w/v). Platelet-rich plasma was separated by centrifugation at $160 \times g$ for 10 min at room temperature and platelet-poor plasma was obtained by centrifugation of the rest of blood at $1800 \times g$ for 10 min at room temperature. The platelet density of platelet-rich plasma was adjusted to 200 000 (human) and 400 000 (guinea pig) platelets/mm^3 with platelet-poor plasma. Platelet aggregation was measured with an aggregometer (model PAT-6A, Niko Bioscience, Japan). Aliquots of platelet-rich plasma were prewarmed at 37°C for 2 min before an aggregating agent was added. The aggregating agents used were U-46619 (human, 2 μM final concentration; guinea pig, 4 μM final concentration), arachidonic acid (guinea pig, 100 μM final concentration), collagen (human and guinea pig, 2 μg/ml final concentration) and ADP (human, 8 μM final concentration; guinea pig, 3 μM final concentration). The changes in light transmission

occurring during platelet aggregation were recorded continuously. The pIC_{50} values (negative logarithm of 50% inhibiting concentration) for test drugs were calculated graphically.

2.5. Platelet aggregation after oral drug administration

Guinea pigs were fasted for 24 h. After fasting, test drugs were given orally in a volume of 1 ml/200 g of body weight. Under ether anesthesia, blood was withdrawn from the abdominal aorta 1 h after oral administration. Platelet aggregation was measured by the method as described in Section 2.4 and the ED_{50} values (50% effective dose) of the test drugs were calculated from the probit curve.

To examine the duration of the inhibitory effects of the test drugs on platelet aggregation, the drugs were given orally 1, 2, 4, 8 and 24 h prior to measurements of platelet aggregation.

2.6. U-46619-induced pulmonary thromboembolism in mice

The effects of the test drugs on U-46619-induced pulmonary thromboembolism in mice was examined by a modification of the procedure reported by Harris et al. (1992). Male ICR mice were fasted for 24 h. Test drugs were given orally in a volume of 100 μl/20 g of body weight 1 h prior to U-46619 (2 mg/kg, i.v.) challenge. Thirty minutes after U-46619 challenge, drug efficacy was estimated as the percentage lethality (number of mice dead/total number of mice × 100).

To examine the duration of the protective effect of Z-335, this drug was given orally 3 and 24 h prior to U-46619 challenge.

2.7. Statistical analysis

Results are expressed as the mean ± S.E.M. and statistical significance was determined by Student's *t*-test and the chi-square test. Probability values of less than 0.05 were considered to be significant.

3. Results

3.1. Drug binding to thromboxane A_2 receptors of human and guinea pig platelets

The specific binding site for [^{3}H]SQ-29548 in washed human platelets was determined by Scatchard analysis to be a single class binding site ($K_d = 4.29 \pm 0.10$ nM; $B_{max} = 193.1 \pm 3.7$ fmol/10^8 platelets). Z-335, vapiprost and daltroban concentration-dependently inhibited [^{3}H]SQ-29548 binding to washed human platelets. The IC_{50} values of Z-335 and daltroban were 29.9 ± 3.1 nM and 86.0 ± 2.8 nM, respectively and the slope factors did not differ from unity. Although the IC_{50} value of vapiprost was 21.7 ± 1.8 nM, its slope factor (1.33 ± 0.03) did differ from unity significantly ($P < 0.05$). From a comparison of the IC_{50} values of the test drugs, the rank order was vapiprost > Z-335 > daltroban (Fig. 2 and Table 1).

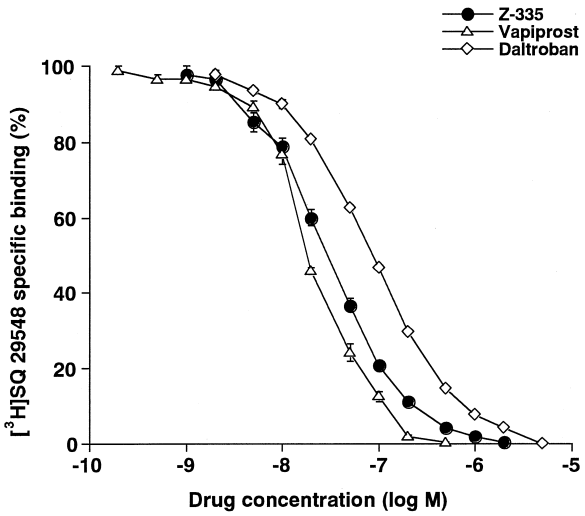

Fig. 2. Dose–response curves of Z-335, vapiprost and daltroban on [^{3}H]SQ-29548 binding to human platelets. Results are expressed as the mean ± S.E.M. ($n = 4$).

The specific binding site for [^{3}H]SQ-29548 in guinea pig platelet membranes was determined by Scatchard analysis to be a single class binding site, as it was for human platelets ($K_d = 8.2 \pm 0.9$ nM; $B_{max} = 2030 \pm 200$ fmol/mg protein). Z-335, vapiprost and daltroban concentration-dependently inhibited [^{3}H]SQ-29548 binding to guinea pig platelet membranes. The IC_{50} values of Z-335, vapiprost and daltroban were 32.5 ± 1.7 nM, 3.69 ± 0.34 nM and 293 ± 5 nM, respectively. From a comparison of the IC_{50} values of the test drugs, the rank order was vapiprost > Z-335 > daltroban (Table 1).

3.2. Inhibitory effect of Z-335 on aggregation of human and guinea pig platelets in vitro

Z-335, vapiprost and daltroban inhibited U-46619- and collagen-induced human platelet aggregation. The pIC_{50}

Table 1
Drug binding to receptors on human platelets and guinea pig platelet membranes

Species	Drug	IC_{50} (nM)	Slope factor
Human	Z-335	29.9 ± 3.1	1.09 ± 0.05
	Vapiprost	21.7 ± 1.8	1.33 ± 0.03[a]
	Daltroban	86.0 ± 2.8	1.01 ± 0.02
Guinea pig	Z-335	32.5 ± 1.7	1.07 ± 0.02
	Vapiprost	3.69 ± 0.34	1.22 ± 0.11
	Daltroban	293 ± 5	1.02 ± 0.02

Results are expressed as the mean ± S.E.M. ($n = 4$).
[a] $P < 0.05$, significantly different from unity (Student's *t*-test).

values of Z-335 were 6.37 ± 0.10 and 5.50 ± 0.22, respectively. The potency of Z-335 was nearly equal to that of daltroban and was about 10 times weaker than that of vapiprost. However, these thromboxane A_2 receptor antagonists did not inhibit ADP-induced platelet aggregation ($pIC_{50} < 4.00$). Cilostazol weakly inhibited U-46619-, collagen- and ADP-induced human platelet aggregation. The pIC_{50} values of cilostazol were 4.85 ± 0.22, 4.94 ± 0.20 and 4.44 ± 0.05, respectively (Table 2).

Z-335, vapiprost and daltroban also inhibited U-46619-, arachidonic acid- and collagen-induced guinea pig platelet aggregation. The pIC_{50} values of Z-335 were 6.22 ± 0.07, 6.63 ± 0.02 and 5.97 ± 0.10, respectively. The potency of Z-335 was nearly equal to that of daltroban and was > 100 times weaker than that of vapiprost. However, these TXA_2 antagonists did not inhibit ADP-induced platelet aggregation ($pIC_{50} < 4.00$). Cilostazol inhibited arachidonic acid and collagen-induced guinea pig platelet aggregation. The pIC_{50} values of cilostazol were 6.57 ± 0.07 and 4.24 ± 0.09, respectively (Table 3).

From a comparison of the pIC_{50} values of the test drugs, the rank order was vapiprost > Z-335 ≥ daltroban > cilostazol.

3.3. Inhibitory effect of Z-335 on aggregation of guinea pig platelets ex vivo

Oral administration of Z-335, vapiprost and daltroban to guinea pigs inhibited U-46619-, arachidonic acid- and collagen-induced platelet aggregation and the ED_{50} values of Z-335 were 0.21 mg/kg (0.14–0.32 mg/kg, 95% confidence limits), 0.22 mg/kg (0.14–0.32 mg/kg, 95% confidence limits) and 0.73 mg/kg (0.51–1.14 mg/kg, 95% confidence limits), respectively. Z-335 was about 3–8 times more potent than daltroban and 5–10 times weaker than vapiprost. However, these test drugs were ineffective against ADP-induced platelet aggregation ex vivo (ED_{50} > 100 mg/kg, p.o.). Cilostazol (100 mg/kg, p.o.) given orally failed to inhibit platelet aggregation induced by U-46619, arachidonic acid or collagen (Table 4).

Table 2
Inhibitory effects of Z-335, vapiprost, daltroban and cilostazol on human platelet aggregation in vitro

Species	Agonists (final concentration)	pIC_{50}			
		Z-335	Vapiprost	Daltroban	Cilostazol
Human	U-46619 (2 μM)	6.37 ± 0.10	7.77 ± 0.10	6.33 ± 0.09	4.85 ± 0.22
	Collagen (2 μg/ml)	5.50 ± 0.22	6.98 ± 0.28	5.35 ± 0.15	4.94 ± 0.20
	ADP (8 μM)	< 4.00	< 4.00	< 4.00	4.44 ± 0.05

Results are expressed as the mean ± S.E.M. ($n = 3$).

Table 3
Inhibitory effects of Z-335, vapiprost, daltroban and cilostazol on guinea pig platelet aggregation in vitro

Species	Agonists (final concentration)	pIC_{50}			
		Z-335	Vapiprost	Daltroban	Cilostazol
Guinea pig	U-46619 (4 μM)	6.22 ± 0.07	8.16 ± 0.03	6.05 ± 0.09	< 4.00
	Arachidonic acid (100 μM)	6.63 ± 0.02	10.46 ± 0.07	6.36 ± 0.04	6.57 ± 0.07
	Collagen (2 μg/ml)	5.97 ± 0.10	8.34 ± 0.07	5.89 ± 0.18	4.24 ± 0.09
	ADP (3 μM)	< 4.00	< 4.00	< 4.00	< 4.00

Results are expressed as the mean ± S.E.M. ($n = 5–8$).

Table 4
Inhibitory effects of Z-335, vapiprost, daltroban and cilostazol on guinea pig platelet aggregation after oral administration

Agonists (final concentration)	ED_{50} (mg/kg, p.o.)			
	Z-335	Vapiprost	Daltroban	Cilostazol
U-46619 (4 μM)	0.21 (0.14–0.32)	0.03 (0.02–0.03)	1.01 (0.70–1.44)	> 100
Arachidonic acid (100 μM)	0.22 (0.14–0.32)	0.02 (0.02–0.03)	0.7 (0.49–1.00)	> 100
Collagen (2 μg/ml)	0.73 (0.51–1.14)	0.16 (0.09–0.27)	5.62 (3.77–8.40)	> 100

Results are expressed as the mean ± S.E.M. ($n = 4–6$). Values in parentheses are the 95% confidence limits.

From a comparison of the ED_{50} values of the test drugs, the rank order was vapiprost > Z-335 > daltroban > cilostazol.

3.4. Duration of inhibition of platelet aggregation in guinea pigs

Oral administration of Z-335 (0.3 and 1 mg/kg, p.o.) inhibited U-46619-induced platelet aggregation for 24 h in guinea pigs. Although vapiprost at 1 and 3 mg/kg (p.o.) inhibited U-46619-induced platelet aggregation for 24 h, the 0.3 mg/kg (p.o.) dose failed to inhibit it for 24 h. Z-335 (3 and 10 mg/kg, p.o.) and vapiprost (3 mg/kg, p.o.) also inhibited collagen-induced platelet aggregation

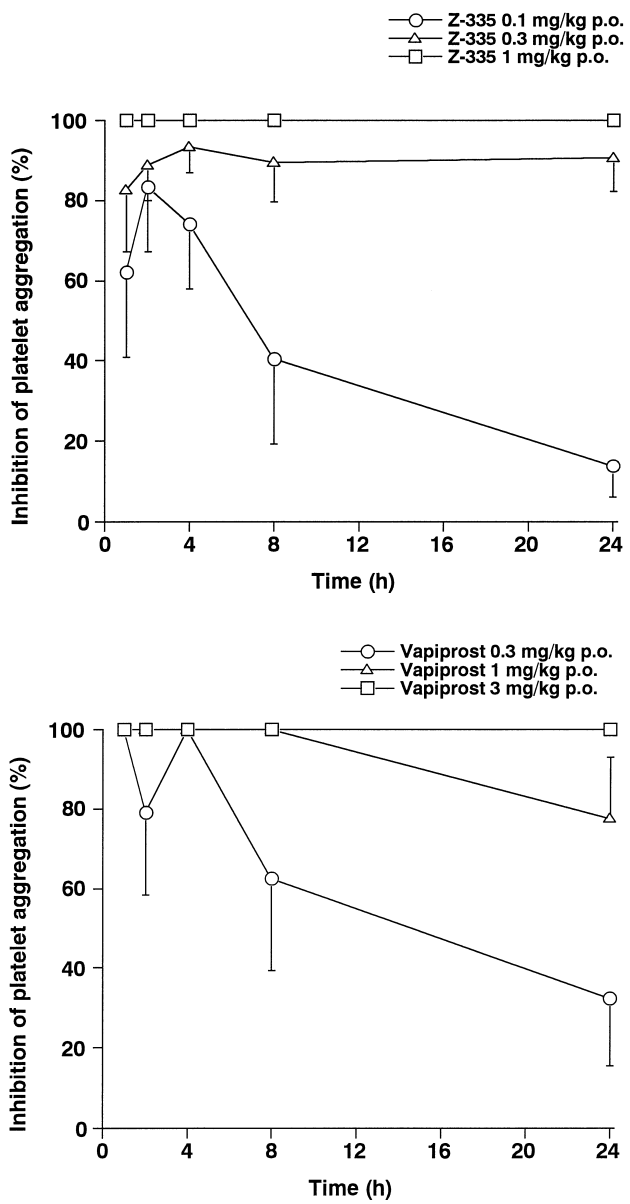

Fig. 3. Duration of the inhibitory effects of Z-335 and vapiprost on U-46619-induced platelet aggregation in guinea pigs after oral administration. Results are expressed as the mean ± S.E.M. ($n = 5–6$).

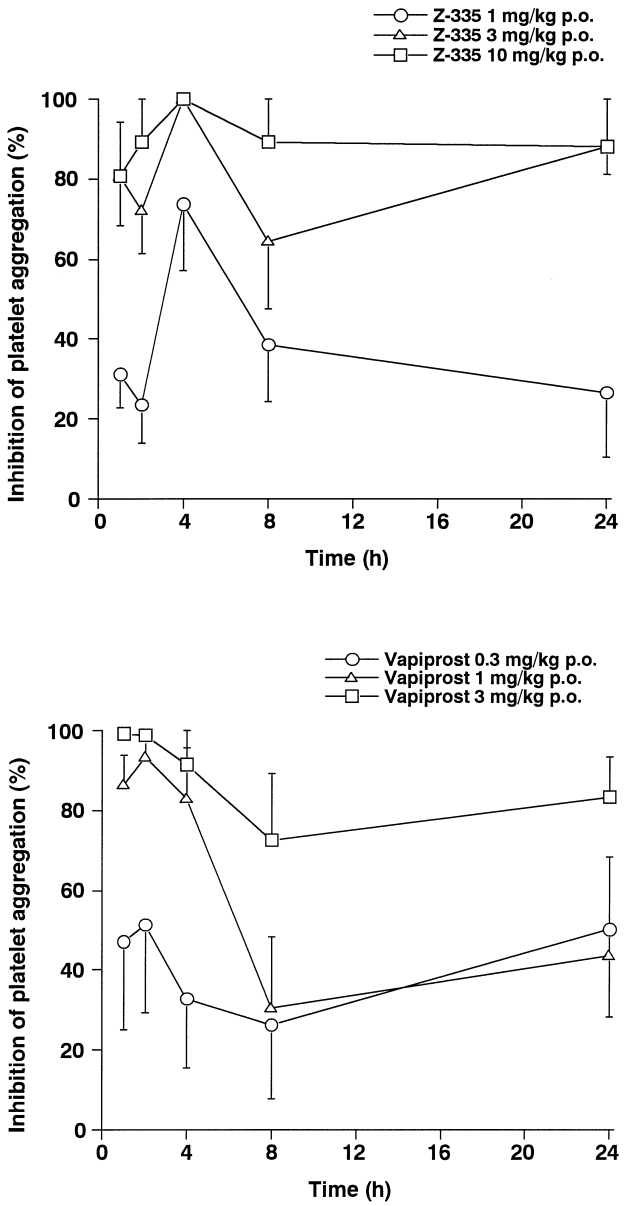

Fig. 4. Duration of the inhibitory effects of Z-335 and vapiprost on collagen-induced platelet aggregation in guinea pigs after oral administration. Results are expressed as the mean ± S.E.M. ($n = 5–6$).

Table 5
Protective effects of Z-335, vapiprost, daltroban and cilostazol on U-46619-induced pulmonary thromboembolism in vivo

Drugs	Total number of mice	Number of mice dead	Lethality (%)
Vehicle	30	27	90
Z-335 0.03 mg/kg, p.o.	20	15	75
0.1 mg/kg, p.o.	20	4	20[a]
0.3 mg/kg, p.o.	19	1	5[a]
Cilostazol 100 mg/kg, p.o.	20	4	20[a]
Vehicle	30	26	87
Vapiprost 10 mg/kg, p.o.	20	12	60[a]
Daltroban 1 mg/kg, p.o.	20	5	25[a]

[a] $P < 0.05$, significantly different from the vehicle-treated group (chi-square test).

Table 6
Duration of the protective effect of Z-335 on U-46619-induced pulmonary thromboembolism in vivo

Time after drug administration	Drugs	Total number of mice	Number of mice dead	Lethality (%)
3 h	Vehicle	20	16	80
	Z-335 0.3 mg/kg, p.o.	20	2	10[a]
	3 mg/kg, p.o.	20	0	0[a]
24 h	Vehicle	19	16	84
	Z-335 0.3 mg/kg, p.o.	20	2	10[a]
	3 mg/kg, p.o.	20	0	0[a]

[a] $P < 0.05$, significantly different from the vehicle-treated group (chi-square test).

of guinea pigs for 24 h. Daltroban (10 mg/kg, p.o.), but not cilostazol (100 mg/kg, p.o.), inhibited U-46619-induced platelet aggregation for 24 h (data not shown). When Z-335 or vapiprost was given orally to guinea pigs at the dose of 0.3 mg/kg, the inhibitory effect of Z-335 on U-46619-induced platelet aggregation continued longer than that of vapiprost (Figs. 3 and 4).

3.5. Inhibitory effect of Z-335 on U-46619-induced pulmonary thromboembolism in vivo

Intravenous injection of U-46619 (2 mg/kg) consistently caused about 90% lethality in vehicle-treated groups. Pretreatment with Z-335 (0.03, 0.1 and 0.3 mg/kg, p.o.) prior to U-46619 challenge dose-dependently reduced the lethality and the dose of 0.3 mg/kg (p.o.) reduced it to 5%. Vapiprost (10 mg/kg, p.o.), daltroban (1 mg/kg, p.o.) and cilostazol (100 mg/kg, p.o.) reduced the lethality to 60%, 25% and 20%, respectively (Table 5). Moreover, Z-335 (0.3 and 3 mg/kg, p.o.) protected the animals from sudden death due to U-46619 challenge for 24 h (Table 6).

4. Discussion

This study was designed to investigate the in vitro, ex vivo and in vivo pharmacological characteristics of Z-335, a new indan derivative. Z-335 inhibited specific binding of [^{3}H]SQ-29548 to human platelets and guinea pig platelet membranes. This result indicates that Z-335 acts at thromboxane A_2 receptor sites, exhibiting thromboxane A_2 receptor antagonism. The slope factors of Z-335 and vapiprost were 1.09 and 1.33 under the present conditions, suggesting that the binding site of Z-335 on human platelets differs from that of vapiprost. Vapiprost has been reported to bind to two isoforms of the thromboxane A_2 receptor (Dorn, 1989; Takahara et al., 1990; Hirata et al., 1996) and our finding that the binding site of vapiprost on human platelets was not a single class binding site is supported by these reports.

The antiplatelet effect of Z-335 on aggregation of human and guinea pig platelets in vitro was nearly equal to that of daltroban and was about >10 times weaker than that of vapiprost. In the ex vivo study, Z-335 was about 3–8 times more potent than daltroban and was about 10 times weaker than vapiprost. Z-335 probably exerts its antiplatelet action via platelet thromboxane A_2 receptor antagonism. It is important that the antiplatelet effect of Z-335 on thromboxane A_2 receptor-mediated platelet aggregation lasted for 24 h in guinea pigs. Indeed, this effect of Z-335 on U-46619-induced platelet aggregation lasted longer than that of vapiprost when Z-335 was given orally to guinea pigs at the same dose (0.3 mg/kg) as vapiprost. The bioavailability of Z-335 in guinea pigs may be superior to that of vapiprost and daltroban. Although the precise mechanism by which Z-335 inhibits guinea pig platelet aggregation for 24 h remains to be elucidated, these results suggest strongly that Z-335 is a potent, orally active and long-lasting thromboxane A_2 receptor antagonist, which may be more useful as an antiplatelet drug than vapiprost and daltroban.

Finally, we tested the antithrombotic effect of Z-335 in a mouse U-46619-induced pulmonary thromboembolism model. Injecting U-46619 into mice caused sudden death as a result of severe pulmonary thromboembolism (Patscheke et al., 1984; Darius and Lefer, 1985; Kosakai et al., 1993). Z-335 pretreatment strongly protected the animals from sudden death due to U-46619 challenge and the protective effect of this drug was greater than those of vapiprost and daltroban. In particular, Z-335 (0.3 and 3 mg/kg, p.o.) strongly protected the animals from sudden death due to U-46619 challenge for 24 h. Z-335 probably exerts its protective action in mice via platelet and vascular smooth muscle cell thromboxane A_2 receptor antagonism. Indeed, Z-335 is a more potent inhibitor of U-46619-induced vasoconstriction in isolated rat aorta than vapiprost or daltroban (unpublished data). The bioavailability of Z-335 in mice, as well as in guinea pigs, may be superior to that of vapiprost and daltroban. Z-335 may be more useful as an orally active antithrombotic drug than vapiprost and daltroban.

Cilostazol is a type III-phosphodiesterase inhibitor, which has antiplatelet and vasodilator actions (Tanaka et al., 1988; Igawa et al., 1990; Saitoh et al., 1993). In this study, cilostazol weakly inhibited aggregation of human and guinea pig platelets in vitro and oral administration of

this drug failed to demonstrate an antiplatelet action in guinea pigs. However, this drug clearly reduced U-46619-induced sudden death in mice. Cilostazol has been reported to prevent sudden death induced by ADP or collagen in mice (Kimura et al., 1985). The protective effect of cilostazol in the mouse U-46619-induced pulmonary thromboembolism model may be due to the antiplatelet and the vasodilator actions of this drug, which may differ somewhat among species. The antiplatelet and antithrombotic potencies of this drug were weaker than those of Z-335.

In summary, we have demonstrated that Z-335 is a potent, orally active and long-lasting thromboxane A_2 receptor antagonist which may be more useful as an antiplatelet drug than vapiprost, daltroban and cilostazol. Our findings suggest strongly that Z-335 may be beneficial in the treatment of vascular thrombosis which involves in platelet activation and vasoconstriction mediated by thromboxane A_2 receptor activation.

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