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Z-335, a new thromboxane A_2 receptor antagonist, prevents arterial thrombosis induced by ferric chloride in rats

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Abstract

We examined the antithrombotic effect of Z-335 ((±)-sodium [2-(4-chlorophenylsulfonylaminomethyl)indan-5-yl]acetate monohydrate), an orally active thromboxane A_2 receptor (TP-receptor) antagonist that ameliorates experimental gangrene, using a rat arterial thrombosis model. The thrombi were induced by topical application of 50% ferric chloride solution to the rats abdominal artery. Z-335 (0.3–3 mg/kg, p.o.) inhibited thrombus formation in a dose-dependent manner. The antithrombotic effect of Z-335 (1 and 3 mg/kg, p.o.) was almost equivalent with that of cilostazol (100 mg/kg, p.o.), a selective phosphodiesterase type III inhibitor. The effect of Z-335 (3 mg/kg, p.o.), but not cilostazol, persisted for 16 h. Z-335, but not cilostazol, inhibited platelet aggregation induced by U-46619 (a TP-receptor agonist, 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$) for 16 h in rat whole blood. Histopathological examination also revealed that Z-335 prevented ferric chloride-induced thrombus formation. These results suggest that Z-335 may prevent ferric chloride-induced arterial thrombosis through its antiplatelet action by blocking TP-receptor activation.

Keywords: Thromboxane A_2; Arterial thrombosis; Z-335; Cilostazol

1. Introduction

Thromboxane A_2, the predominant arachidonate metabolite which evokes platelet aggregation and vascular smooth muscle constriction, plays an important role in arterial thrombosis. Thromboxane A_2 is also a proliferative agent, and thus is considered to be a pathological factor in cardiovascular disease (Halushka et al., 1995).

A number of arterial thrombosis models have been developed for evaluating novel antithrombotic agents. A rat model of ferric chloride-induced arterial thrombosis has been demonstrated to be useful for assessment of antithrombotic activity in vivo (Broersma et al., 1991). Topical application of ferric chloride to the rat carotid artery denudes the vascular endothelium, resulting in the formation of an occlusive thrombus composed of both platelets and fibrin (Kurz et al., 1990). Aspirin, a widely accepted antiplatelet drug that mainly inhibits thromboxane A_2 production, has little effect on ferric chloride-induced thrombi (Lockyer and Kambayashi, 1999), possibly because of inhibition of prostacyclin, which exerts antithrombotic activity in rats. Therefore, further investigation of the involvement of thromboxane A_2 in this model is of interest.

Z-335 ((±)-sodium [2-(4-chlorophenylsulfonylaminomethyl)indan-5-yl]acetate monohydrate) is an orally active thromboxane A_2 receptor (TP-receptor) antagonist. Z-335 inhibits the specific binding of [^{3}H]SQ-29548 to human platelets with a IC_{50} value (50% inhibiting concentration) of 29.9 nM and U-46619 (a TP-receptor agonist)-induced human platelet aggregation in vitro with a pIC_{50} value (negative logarithm of 50% inhibitory concentration) of 6.37. Z-335 also ameliorates experimental hind limb gangrene in rats (Tanaka et al., 1998a,b; 1999), and is expected to be useful for treatment of peripheral vascular disease. However, its antithrombotic activity has not been fully investigated.

The primary aim of this study was to examine the antithrombotic effect of Z-335 in arterial thrombosis induced by topical application of ferric chloride. Cilostazol, a phosphodiesterase type III inhibitor that shows antiplatelet and vasodilator properties, is used clinically as an antithrombotic drug in the treatment of peripheral vascular disease (Kimura et al., 1985; Narita et al., 1995). There-

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fore, we also compared the antithrombotic effect of Z-335 with that of cilostazol in this model.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Japan), weighing 250–300 g were housed in a temperature-controlled room before being used in the present experiments. All experiments were performed in accordance with the regulations of the Animal Committee of ZERIA Pharmaceutical, conforming to the Guiding Principles of the Japanese Pharmacological Society.

2.2. Ferric chloride-induced rat arterial thrombosis

The experiments were carried out according to the modification of the method described by Kurz et al. (1990). Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). After an abdominal midline incision, the abdominal aorta was exposed carefully. A filter paper disk (diameter 8 mm) saturated with 50% (w/v) ferric chloride solution was placed on the surface of the artery for 10 min. The artery was isolated 10 min after removing the disk and then opened lengthwise. The thrombus was scraped out and placed on filter paper to remove any water, and its wet weight was measured immediately. Z-335 (0.1 and 3 mg/kg) and cilostazol (100 mg/kg) were given orally 1 h prior to application of ferric chloride. These drugs were suspended in 0.5% (w/v) methylcellulose solution at a volume of 5 ml/kg.

2.3. Platelet aggregation in rat whole blood ex vivo

Platelet aggregation was measured with a whole blood aggregometer (model 560, Chrono-Log, USA) 1 h after drug administration. Briefly, the rats were anesthetized with ether, and blood was drawn from the abdominal aorta into a syringe containing a 1/10 volume of 100 unit/ml heparin. Heparinized blood (500 μl) was mixed with 490 μl physiological saline in a cuvette maintained at 37°C. Then, 10 μl of U-46619 (500 nM final concentration) was added to the blood sample. The results were expressed as the maximum impedance, 5 min after adding U-46619.

2.4. Duration of antithrombotic and antiplatelet effects

To evaluate the duration of the antithrombotic effects of Z-335 and cilostazol, these drugs were given orally 4, 8 and 16 h prior to ferric chloride application. Then, to examine the duration of antiplatelet activity of these drugs, platelet aggregation was measured 4, 8 and 16 h after drug administration. The measurement of thrombus weight and platelet aggregation was performed according to Sections 2.2 and 2.3, respectively.

2.5. Histopathological examination of ferric chloride-induced arterial thrombosis

Z-335 (1 mg/kg) was given orally 1 h prior to ferric chloride application. At 10 min after removing the disk saturated with ferric chloride solution, the abdominal aorta was isolated and fixed with 10% (v/v) formalin and embedded in paraffin. Sections obtained from the artery were stained with hematoxylin and eosin.

2.6. Drugs

Z-335 ((±)-sodium [2-(4-chlorophenylsulfonylaminomethyl)indan-5-yl]acetate monohydrate), and cilostazol (6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)-butoxy]-3,4-dihydro-2-(1H)-quinolinone), synthesized in our laboratories, were suspended in 0.5% (w/v) methyl cellulose solution. Ferric chloride (Nacalai Tesque, Kyoto, Japan) was dissolved in water and the concentration was expressed in terms of the actual weight of ferric chloride. U-46619 (9,11-dideoxy-9α, 11α-methanoepoxy-prostaglandin $F_{2\alpha}$), a thromboxane A_2 analogue, was purchased from Cayman Chemical (MI, USA). It was dissolved in 99.5% (v/v) ethanol and diluted with physiological saline.

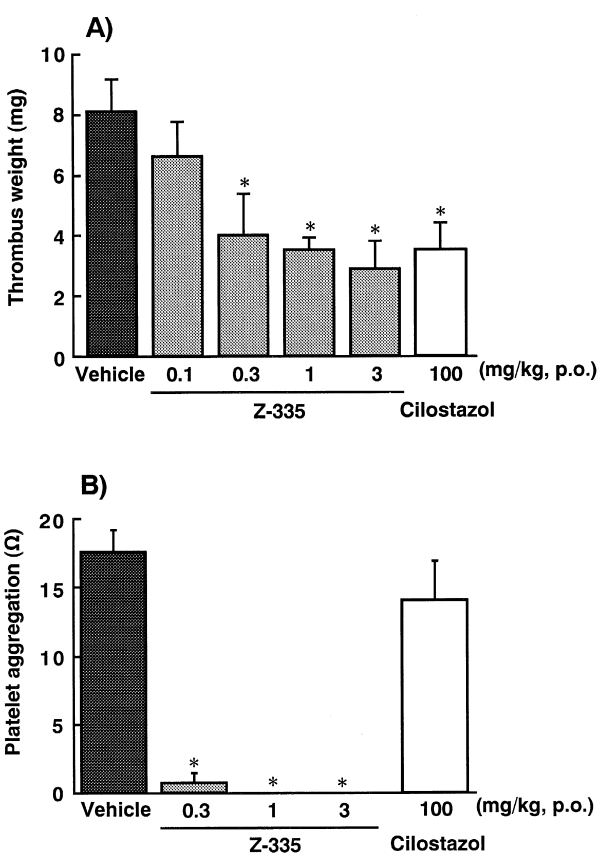

Fig. 1. Effects of Z-335 on thrombus weight (A) and platelet aggregation. (B) Test drugs were given orally 1 h prior to ferric chloride application or measurement of platelet aggregation. Each column represents the mean ± SEM of 6–7 rats. * $P < 0.05$, compared with the vehicle-treated group.

2.7. Statistical analysis

The results are expressed as mean ± SEM. The statistical significance of any difference was evaluated by Dunnett's two-tailed test, following one-way analysis of variance. Differences at $P < 0.05$ were considered significant.

3. Results

3.1. Antithrombotic effect of Z-335 on ferric chloride-induced arterial thrombosis

Topical application of ferric chloride to the abdominal aorta induced thrombi (8.09 ± 1.11 mg) in vehicle-treated rats. Z-335, at oral doses of 0.3, 1 and 3 mg/kg, reduced thrombus weight by 50%, 56% and 64%, respectively. Cilostazol (100 mg/kg, p.o.) also inhibited thrombus formation by 56% (Fig. 1A). The antithrombotic effect of cilostazol was almost equivalent to that of Z-335 (1 and 3 mg/kg, p.o.).

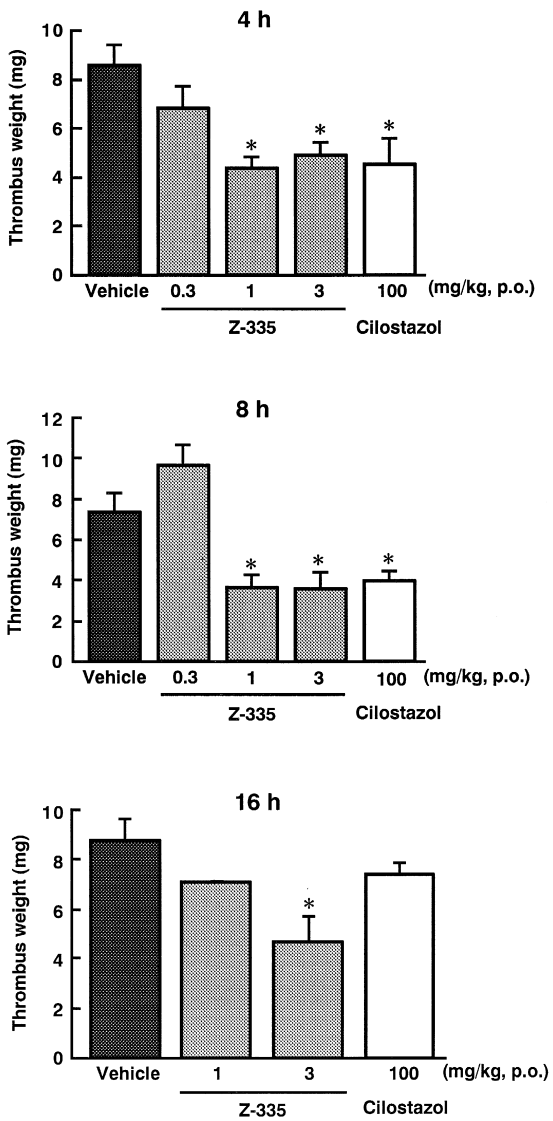

Fig. 2. Duration of antithrombotic effect of Z-335 on ferric chloride-induced arterial thrombosis. Test drugs were given orally 4, 8 or 16 h prior to ferric chloride application. Each column represents the mean ± SEM of 5–7 rats. * $P < 0.05$, compared with the vehicle-treated group.

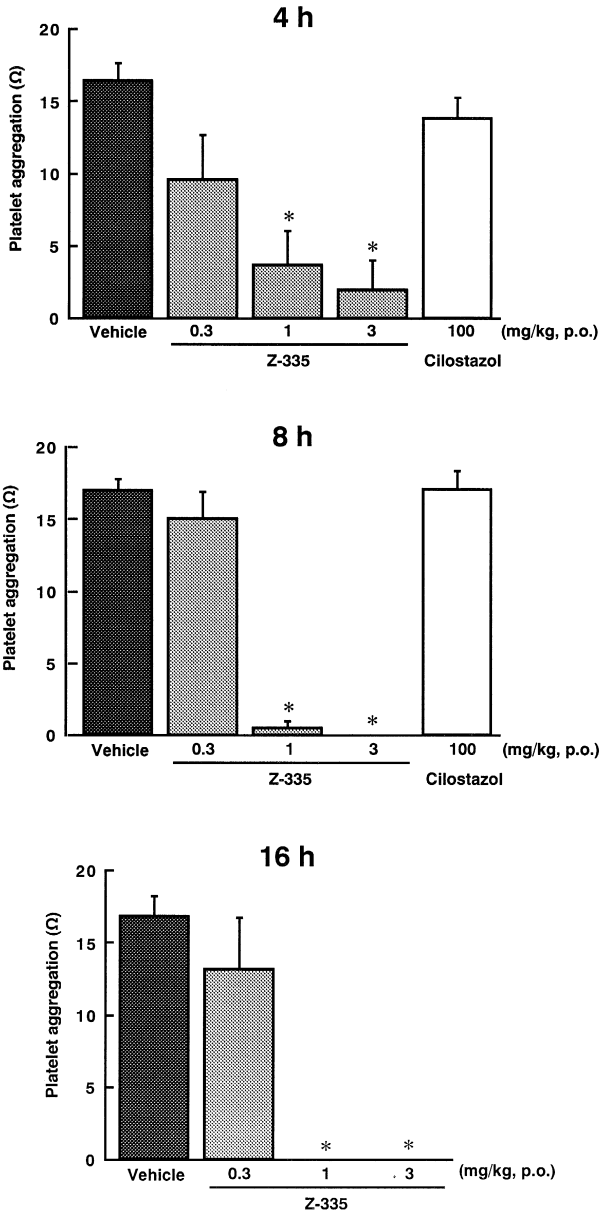

Fig. 3. Duration of antiplatelet effect of Z-335 on platelet aggregation. Test drugs were given orally 4, 8 or 16 h prior to measurement of platelet aggregation. Each column represents the mean ± SEM of 5–6 rats. * $P < 0.05$, compared with the vehicle-treated group.

3.2. Antiplatelet effect of Z-335 ex vivo

Z-335, at an oral dose of 0.3 mg/kg, effectively inhibited U-46619-induced platelet aggregation in rat whole

blood, 1 h after administration, and higher doses of Z-335 (1 and 3 mg/kg, p.o.) abolished platelet aggregation completely. In contrast, cilostazol (100 mg/kg, p.o.) failed to show any antiplatelet activity (Fig. 1B).

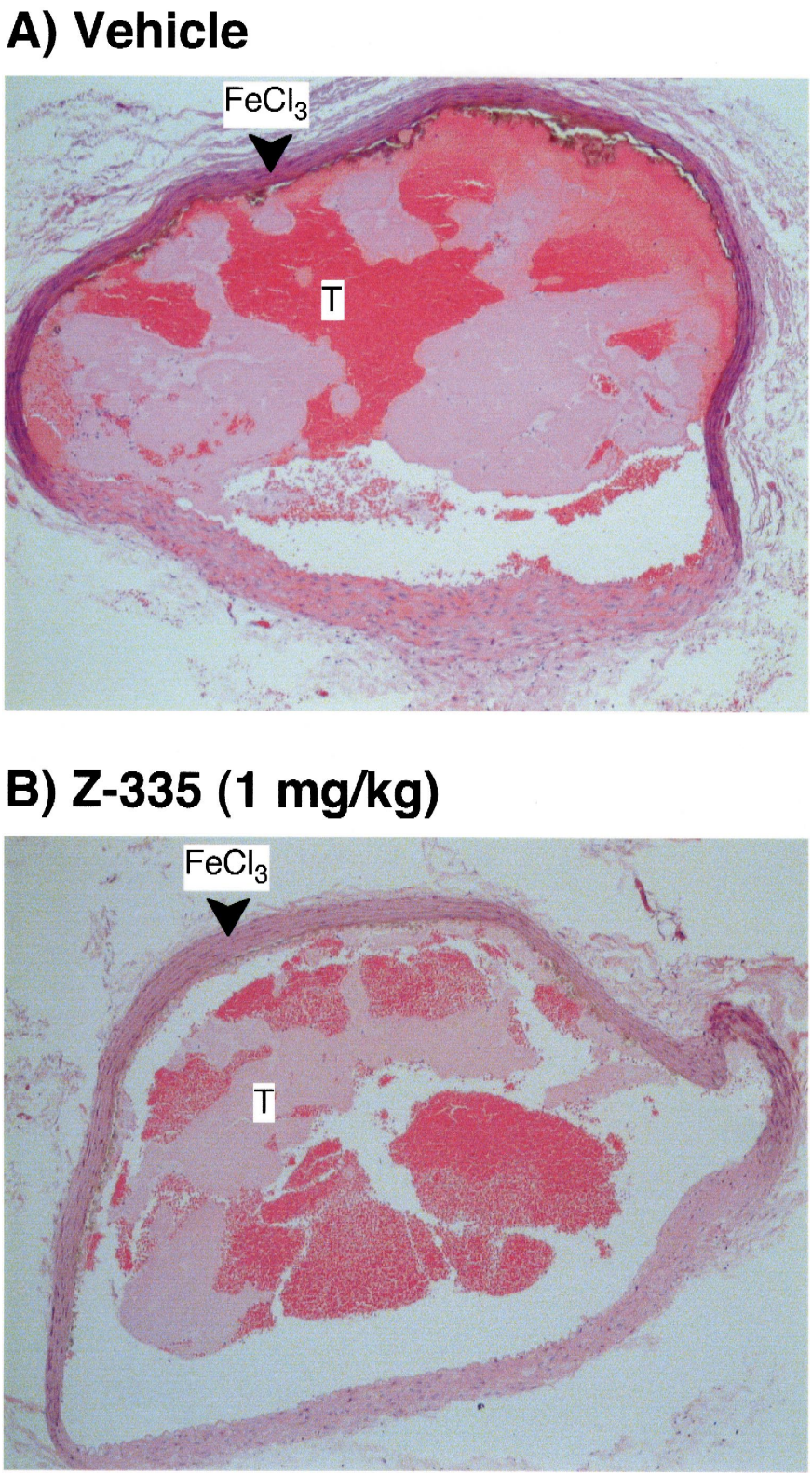

Fig. 4. Light micrographs of ferric chloride-induced thrombi from the vehicle-treated group (A) and Z-335-treated (1 mg/kg, p.o.) group. (B) Z-335 was given orally 1 h prior to ferric chloride application. Ferric chloride was applied to the upper side in the light micrographs. $FeCl_3$, ferric chloride; T, thrombus. Both sections were stained with hematoxylin–eosin (×200).

3.3. Duration of antithrombotic and antiplatelet effects of Z-335

Z-335 (1 and 3 mg/kg, p.o.) reduced thrombus weight significantly when given 4 or 8 h prior to ferric chloride application. The highest dose of Z-335 (3 mg/kg) significantly prevented thrombus formation even at 16 h after oral administration. A similar antithrombotic effect was observed with cilostazol (100 mg/kg, p.o.) for 8 h (Fig. 2).

Z-335 (1 and 3 mg/kg) also effectively inhibited U-46619-induced platelet aggregation when given orally 4, 8 or 16 h prior to blood collection. However, cilostazol (100 mg/kg, p.o.) had no effect against U-46619-induced platelet aggregation, irrespective of the timing of administration (Fig. 3).

3.4. Histopathological examination of ferric chloride-injured artery

Application of ferric chloride to the abdominal aorta induced marked thrombi that were adherent to the vessel wall at the site of ferric chloride contact. Pretreatment with Z-335 (1 mg/kg, p.o.) prevented thrombus formation, but did not prevent vascular injury due to ferric chloride (Fig. 4).

4. Discussion

This study was designed to clarify the antithrombotic effect of Z-335 on ferric chloride-induced arterial thrombosis in rats. This model produces mixed thrombi that are rich in platelets and fibrin (Kurz et al., 1990). Moreover, inhibition of platelet function or the coagulation pathway is partly effective in preventing thrombus formation (Lockyer and Kambayashi, 1999). Thus, the development of thrombi in response to ferric chloride-induced vascular injury seems to be physiologically relevant. In this study, Z-335 clearly prevented thrombus formation following ferric chloride-induced vascular injury, and our histopathological examination also confirmed its antithrombotic effect. Moreover, the antithrombotic activity of Z-335 was almost equivalent to that of cilostazol (100 mg/kg, p.o.). These findings suggest that Z-335, like cilostazol, is effective in preventing acute and mixed-type arterial thrombosis. Our result appears to be supported by a previous report indicating that, in a similar rat model of ferrous chloride-induced carotid artery thrombosis, a selective TP-receptor antagonist, BMS-180291, given intravenously, decreased the weight of arterial thrombi (Schumacher et al., 1993).

The involvement of platelets in this model has been directly indicated by induction of thrombocytopenia with antiplatelet serum (Lockyer and Kambayashi, 1999). To estimate the contribution of the antiplatelet activity of Z-335 and cilostazol to their antithrombotic action, we further examined the activity of these drugs on platelet aggregation induced by U-46619, a thromboxane A_2 analogue. Oral administration of Z-335 markedly inhibited U-46619-induced platelet aggregation in rat whole blood. The doses required to inhibit platelet aggregation closely corresponded to those which suppressed ferric chloride-induced thrombi, suggesting that Z-335 may prevent arterial thrombosis through its antiplatelet action by blocking TP-receptors.

On the other hand, cilostazol failed to inhibit U-46619-induced platelet aggregation. It is generally accepted that cilostazol exerts its antiplatelet and vasodilative action through elevation of cyclic AMP levels in platelets and vascular smooth muscle cells (Okuda et al., 1993). However, the antiplatelet activity of this drug is weaker in rats than in human, dogs and mice (Kimura et al., 1985). Cilostazol (100 mg/kg, p.o.) has been reported to inhibit thrombus formation in a rat model of abdominal aorta thrombosis (Narita et al., 1995). In contrast, Kawasaki et al. (1998) reported that this drug, at the same dose, was ineffective in an electrically induced carotid artery thrombosis in rats. Thus, the antithrombotic activity of cilostazol also seems to differ somewhat among rat thrombosis models. Interestingly, the antiplatelet activity of cilostazol is potentiated by the presence of endothelial cells (Igawa et al., 1990). Although the precise mechanism(s) by which cilostazol exerts its antithrombotic effect in this model is unclear, antithrombotic factors derived from endothelial cells may be partly responsible, in addition to its weak antiplatelet property.

In our previous study, repeated oral administration of Z-335 (10 mg/kg, once daily) for 10 days, ameliorated arachidonic acid-induced hind limb gangrene more efficiently than cilostazol (100 mg/kg, p.o., once daily) in rats (Tanaka et al., 1999). Histopathological examination of the paw injury also demonstrated that Z-335 effectively prevented the formation of occlusive thrombi following vascular injury due to arachidonic acid (Tanaka et al., 1999). Accordingly, we consider that continuous blocking of TP-receptor activation may be important for ameliorating the thrombosis associated with vascular injury. In this study, the antithrombotic action of Z-335 lasted longer than that of cilostazol. Its antiplatelet activity was also more potent than that of cilostazol in the ex vivo study. Thus, Z-335 may be more beneficial than cilostazol as a drug for ameliorating peripheral vascular disease.

We have reported that Z-335 did not prolong rat tail bleeding time (Tanaka et al., 1998b). It still remains uncertain what causes the separation of antithrombotic and bleeding time effects of Z-335. Under our experimental condition, vapiprost (a potent TP-receptor antagonist) and aspirin (a cyclooxygenase inhibitor) also did not affect the tail bleeding time (Tanaka et al., 1998b). Thus, thromboxane A_2 may not be involved in the activation of platelets in rat primary hemostasis.

In conclusion, we have demonstrated that Z-335 prevents ferric chloride-induced arterial thrombosis in rats. This antithrombotic effect may be due to its antiplatelet action by blocking of TP-receptor activation. Thus, thromboxane A_2 may be partly involved in the pathogenesis of ferric chloride-induced arterial thrombosis in rats.

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