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Antithrombotic effects in a rat model of aspirin-insensitive arterial thrombosis of desethyl KBT-3022, the main active metabolite of a new antiplatelet agent, KBT-3022

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Abstract

The antithrombotic effect of desethyl KBT-3022, which is the main active metabolite of the new antiplatelet agent, KBT-3022 (ethyl 2-[4,5-bis(4-methoxyphenyl)thiazol-2-yl] pyrrol-1-ylacetate; a cyclooxygenase inhibitor), was determined using a photochemically induced arterial thrombosis model in the rat femoral artery. Pretreatment with desethyl KBT-3022 (0.1, 0.3 and 1 mg/kg, i.v.) prolonged the time required to achieve thrombotic occlusion in the femoral artery and inhibited collagen-induced platelet aggregation in whole blood ex vivo, each in a dose-dependent manner. In all 6 rats used, particularly at the highest dose (1 mg/kg, i.v.) tested, cyclic variations in blood flow were hardly ever observed and complete cessation of blood flow did not occur during the 30-min observation time. BM-13505 (1, 3 and 10 mg/kg, i.v.), a thromboxane A_2 receptor antagonist, also prolonged the time to occlusion, but cyclic variations in blood flow did occur. On the other hand, aspirin (10 and 30 mg/kg, i.v.) had little effect in terms of preventing thrombosis, although it inhibited collagen-induced platelet aggregation to the same extent as did desethyl KBT-3022. Desethyl KBT-3022 inhibited the thrombin-induced aggregation of washed platelets in a concentration-dependent manner (1–40 μM), whereas aspirin and BM-13505 did not. These findings suggest that the potent antithrombotic effect of desethyl KBT-3022 may be attributable in part to its additional ability to inhibit thrombin-induced platelet aggregation. Accordingly, thromboxane A_2 and thrombin may be important thrombotic mediators in this rat model.

Keywords: Arterial thrombosis; Cyclooxygenase; Thromboxane A_2; Platelet aggregation

1. Introduction

Platelet activation, adhesion and aggregation play a pivotal role in the pathogenesis of arterial thrombosis, and antiplatelet agents such as aspirin have therefore become widely used in the management of thrombotic disorders. The effect of aspirin is attributed to its ability to inhibit platelet cyclooxygenase, thus preventing the formation of thromboxane A_2, a potent platelet activator and vasoconstrictor. However, the activation pathways of other agonists, such as thrombin, are left uninhibited by aspirin (Roth and Calverley, 1994). In addition, aspirin inhibits the synthesis of prostacyclin in the endothelium; this is an undesirable effect as this prostanoid may have potent antiplatelet and vasodilator effects (Roth and Calverley, 1994). This may explain why aspirin is not fully effective clinically.

Ethyl 2-[4,5-bis(4-methoxyphenyl)thiazol-2-yl] pyrrol-1-ylacetate (KBT-3022) is a newly synthesized antiplatelet agent (Yamashita et al., 1990). Desethyl KBT-3022 is its main active metabolite and it inhibits not only cyclooxygenase, but also 5-lipoxygenase (Yamashita et al., 1990). It also inhibits thrombin-induced platelet aggregation (Yamashita et al., 1990). Oral administration of KBT-3022 has been reported to inhibit thromboxane A_2 synthesis in platelets more potently than it does prostacyclin synthesis in the blood vessel wall (Yamashita et al., 1990). There-

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fore, desethyl KBT-3022 can be expected to be more potent in terms of antithrombotic efficacy than aspirin.

Recently, we have developed a photochemically induced thrombosis model in the femoral artery of the rat and guinea-pig (Takiguchi et al., 1992a). This model allows us to determine the efficacy with which antiplatelet agents prevent thrombus formation in response to endothelial injury. This photochemically induced thrombosis model in rats has been demonstrated to be aspirin-insensitive, although thromboxane A_2 plays a primary role in the thrombogenesis (Takiguchi et al., 1992a).

In the present study, we compared the antithrombotic effect of desethyl KBT-3022 with the effects of aspirin and BM-13505, a thromboxane A_2 receptor antagonist, using our photochemically induced thrombosis model in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (SLC, Japan) weighing 240–320 g were used. The animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and body temperature was maintained at 37°C with a heating pad throughout the experiment.

2.2. Photochemically induced thrombosis model

The photochemically induced thrombosis model in rat femoral artery used in our laboratory has been described previously in detail (Takiguchi et al., 1992a). Briefly, the right femoral artery was carefully exposed and a 5-mm segment distal to the inguinal ligament cleared from surrounding tissue. A pulsed Doppler flow probe (PDV-20, Crystal Biotech America, USA) was placed on the artery for monitoring blood flow. The contralateral vein and the right carotid artery were cannulated with polyethylene tubes for drug delivery and for the monitoring of blood pressure and pulse rate, respectively. The arterial blood flow, blood pressure and pulse rate were continuously monitored on a thermal array recorder (WS-681G, Nihon-Kohden, Japan). Transillumination of the right femoral artery with green light (540 nm wavelength) was achieved using a L4487 irradiation apparatus (Hamamatsu Photonics, Japan). The irradiation was directed via an optic fiber positioned 5 mm away from the section of the artery proximal to the flow probe. After establishing baseline blood flow, irradiation was started, and then rose bengal (Wako, Japan) at 15 mg/kg (i.v.) was injected within 1 min. Blood flow was monitored for 30 min after the start of the rose bengal injection. The successful formation of an occlusive thrombus was indicated by a complete cessation of blood flow. The time required to achieve complete occlusion was recorded.

2.3. Collagen-induced platelet aggregation in whole blood (ex vivo)

Blood samples from rats treated with drugs or vehicle were collected from the abdominal aorta into 50 U/ml heparin (9:1, v/v). Platelet aggregation in whole blood was measured using a platelet-counting technique as follows. A 990-μl aliquot of whole blood diluted with saline (1:1, v/v) was pipetted into a siliconized cuvette, with constant stirring at 1000 rpm at 37°C in a whole-blood aggregometer (C550, Chrono-Log, USA): 10 μl of collagen (Hormon-Chemie, Germany) was added to give a final concentration of 1 μg/ml. After 7 min, the number of single platelets was determined using a Haematology Analyzer (MEK-4150, Nihon Kohden, Japan), and platelet aggregation was expressed as the percentage decrease in the platelet count. For each rat, the platelet count just before stimulation was taken as 100%.

2.4. Thrombin-induced aggregation of washed platelets (in vitro)

Blood was collected into 3.8% sodium citrate (9:1, v/v) from the abdominal aorta and centrifuged at $100 \times g$ for 10 min at room temperature to obtain platelet-rich plasma. Platelet-rich plasma was added to the same volume of Tris-buffered saline (25 mM Tris-HCl, 130 mM NaCl, pH 7.4) containing 1.5 mM EDTA and centrifuged at $500 \times g$ for 8 min at room temperature. The pellet was washed with Tris-buffered saline containing 0.1% bovine serum albumin and 0.1% glucose and centrifuged at $500 \times g$ for 8 min at room temperature, twice. Finally, the pellet was resuspended in Tris-buffered saline containing 0.1% bovine serum albumin and 0.1% glucose, and the platelet count was adjusted to 4×10^8 cells/ml.

Platelet aggregation was measured according to Born's turbidimetric method (Born and Cross, 1963) using an aggregometer (Hematracer, Niko Bioscience, Japan). After preincubation with drugs for 10 min, aggregation was induced by 0.4 U/ml thrombin (Mochida, Japan) in the presence of 1.5 mM Ca^{2+}.

2.5. Platelet retention in a collagen-coated-bead column (ex vivo)

Platelet retention was determined as follows. Samples (2.5 ml) of blood treated with drugs or vehicle were taken from the abdominal aorta into a syringe (2.5 ml, TERUMO, Japan) without anticoagulant. Immediately, 1 ml of the blood was transferred into a sample cup containing EDTA-2K. The remainder was pulled through a microadhesion column – which contained plastic beads coated with type I collagen prepared from porcine skin (ISK, Japan) – using a constant-flow pump (ISK, Japan) at 2 ml/min, and collected into a sample cup containing EDTA-2K. Platelet

retention was determined by counting single platelets in blood samples before and after exposure to the collagen-coated-bead column using a Haematology Analyzer (MEK-4150, Nihon Kohden, Japan), and was expressed as the percentage of platelets bound to the collagen.

2.6. Drugs

Desethyl KBT-3022 and BM-13505 were gifts from New Drug Research Laboratories of Kanebo, Japan. Aspirin was purchased from Sigma (USA). All drugs were dissolved in 33% ethanol solution containing 33% polyethylene glycol 400 and, when used, administered intravenously 5 min before the injection of rose bengal or the collection of blood. In the experiment on thrombin-induced platelet aggregation, desethyl KBT-3022, BM-13505 and aspirin were dissolved in dimethyl sulfoxide and diluted to the desired concentration with TBS (final concentration of dimethyl sulfoxide 0.4%).

2.7. Statistical analysis

The results are expressed as means ± S.E. Time-to-occlusion values were analyzed using the non-parametric Kruskal-Wallis test followed by Dunnett's test. When complete occlusion did not occur during the 30-min observation time, the time to occlusion was taken as 30 min. Other data were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's test. Results were considered to show a significant difference at $P < 0.05$.

3. Results

3.1. Effects of antiplatelet agents on the photochemically induced thrombosis model

Typical changes in blood flow in the irradiated femoral artery and in arterial blood pressure and pulse rate after the injection of rose bengal under green light irradiation are shown in Fig. 1. The inhibitory effects of antiplatelet agents on thrombus formation are summarized in Fig. 2. In the vehicle-treated group, the irradiated femoral artery was completely occluded in 6.50 ± 0.88 min ($n = 7$) after the injection of rose bengal under green light irradiation without any prolonged changes in blood pressure or pulse rate. Treatment with desethyl KBT-3022 at 0.1, 0.3 and 1 mg/kg, i.v. (administered 5 min before rose bengal injection) dose-dependently prolonged the time to vasculature occlusion, the effect being significant at doses of 0.3 and 1 mg/kg. In all 6 rats used, particularly at the highest dose of desethyl KBT-3022 (1 mg/kg), cyclic variations in blood flow were rarely, if ever, observed and complete cessation of the blood flow did not occur during the

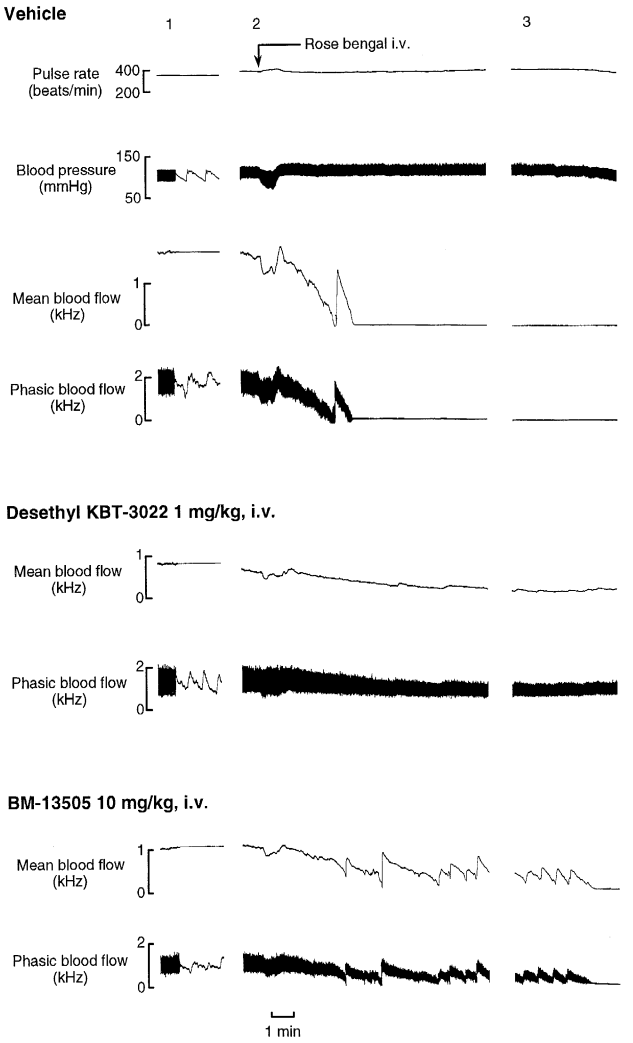

Fig. 1. Representative tracings of phasic and mean blood flow in an irradiated femoral artery, with heart rate and arterial blood pressure, before and after injection of rose bengal under green light irradiation. (1) Baseline records; (2) immediately before and after injection of rose bengal (15 mg/kg, i.v.); (3) 30 min after the initiation of the photochemical reaction. In the middle and lower panels, desethyl KBT-3022 and BM-13505, respectively, were administered intravenously 5 min before the initiation of the photochemical reaction.

30-min observation time (Fig. 1, middle panel), although blood flow did gradually decrease. BM-13505 (at 1, 3 and 10 mg/kg) also prolonged the time to vasculature occlusion in a dose-dependent manner and, at a dose of 10 mg/kg, the effect was statistically significant. However, cyclic variations in blood flow were observed in the group of rats receiving BM-13505 (Fig. 1, lower panel). Aspirin at 10 and 30 mg/kg, i.v., failed to inhibit thrombus formation in this model, as we have already reported elsewhere (Massad et al., 1987; Takiguchi et al., 1992a).

None of the drugs, even at the highest dose tested, caused any changes in haemodynamics, i.e., in blood pressure, pulse rate or resting blood flow in the femoral artery (data not shown).

Table 1
Effects of desethyl KBT-3022, aspirin and BM-13505 on ex vivo collagen-induced platelet aggregation in rat whole blood

Drugs (mg/kg, i.v.)		*n*	Platelet aggregation (%)
Vehicle		6	70.7 ± 2.9
Desethyl KBT-3022	0.1	5	48.3 ± 3.5 [a]
	0.3	5	37.4 ± 3.0 [a]
	1	5	28.5 ± 4.8 [a]
Aspirin	10	5	44.5 ± 4.7 [a]
	30	5	23.9 ± 3.3 [a]
BM-13505	1	5	52.6 ± 3.9 [a]
	3	5	44.8 ± 5.1 [a]
	10	5	41.8 ± 0.9 [a]

Drugs were administered intravenously 5 min before the collection of blood. Data are expressed as means ± S.E.M. The concentration of collagen used was 1 μg/ml.
[a] $P < 0.01$ indicates significance by Dunnett's test.

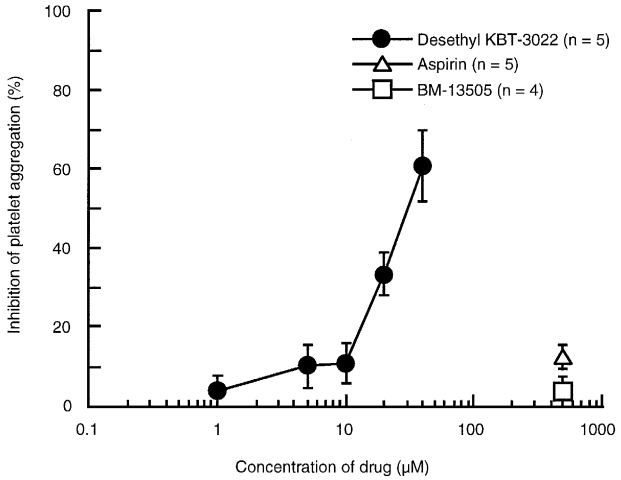

Fig. 3. Effects of desethyl KBT-3022, aspirin and BM-13505 on thrombin-induced aggregation of washed platelets in rats in vitro. Desethyl KBT-3022, aspirin or BM-13505 was added 10 min before addition of thrombin (0.40 U/ml). Each point represents mean ± S.E.

3.2. Effects of antiplatelet agents on collagen-induced platelet aggregation in whole blood (ex vivo)

Effects of the antiplatelet agents on the ex vivo platelet aggregation induced by collagen (1 μg/ml) in whole blood are shown in Table 1. Desethyl KBT-3022 (0.1, 0.3 and 1 mg/kg), aspirin (10 and 30 mg/kg) and BM-13505 (1, 3 and 10 mg/kg) all dose-dependently and significantly inhibited collagen-induced platelet aggregation in whole blood, and to much the same extent. There were no significant differences between the vehicle-treated group and any of the drug-treated groups in terms of the numbers of platelets in whole blood 5 min after drug administration (data not shown).

3.3. Effects of antiplatelet agents on thrombin-induced aggregation in washed platelets (in vitro)

The results of in vitro experiments on thrombin (0.4 U/ml)-induced platelet aggregation are shown in Fig. 3. Desethyl KBT-3022 inhibited such aggregation in a con-

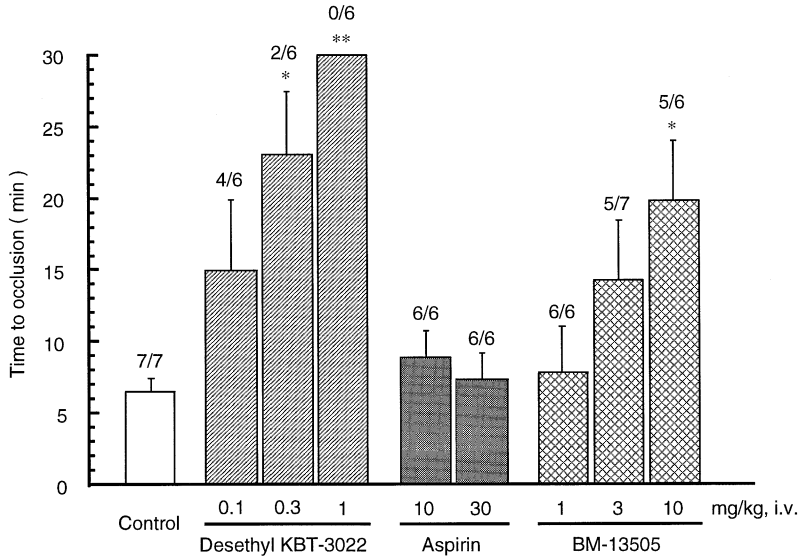

Fig. 2. Effects of desethyl KBT-3022, aspirin and BM-13505 on photochemically induced thrombosis in rats. Desethyl KBT-3022, aspirin or BM-13505 was administered intravenously at the doses shown, 5 min before the initiation of the photochemical reaction. Numbers above each column indicate occlusion rate. Time to occlusion in the non-occluded animal was taken as 30 min. Each column represents mean ± S.E. * $P < 0.05$, ** $P < 0.01$ indicate significant difference from vehicle-treated, 'control', group (non-parametric Kruskal-Wallis test followed by Dunnett's test).

Table 2
Effects of desethyl KBT-3022 and aspirin on platelet retention in a collagen-coated-bead column in rats

Drugs (mg/kg, i.v.)		*n*	Platelet count ($\times 10^4$ cells/μl)	Platelet retention (%)
Vehicle		8	98.3 ± 2.9	31.5 ± 3.3
Desethyl KBT-3022	1	8	100.1 ± 2.3	27.6 ± 2.4
Aspirin	30	8	96.3 ± 2.9	31.0 ± 3.9

Drugs were administered intravenously 5 min before the collection of blood. Data are expressed as means ± S.E.M.

centration-dependent manner (1–40 μM). In contrast, aspirin and BM-13505 had no effect, even at 500 μM.

3.4. Effects of antiplatelet agents on platelet retention in a collagen-coated-bead column (ex vivo)

As shown in Table 2, in the vehicle-treated group platelet retention was 31.5 ± 3.3% ($n = 8$). Pretreatment with desethyl KBT-3022 at 1 mg/kg seemed to somewhat inhibit platelet retention, but the effect did not reach significance. Aspirin at 30 mg/kg was ineffective.

4. Discussion

In this study, the antithrombotic effect of desethyl KBT-3022 was evaluated in our photochemically induced thrombosis model in rats and compared with the effects of aspirin and of BM-13505, a selective thromboxane A_2 receptor antagonist. Aspirin at 10 and 30 mg/kg, i.v., failed to prolong the time to thrombotic occlusion in the femoral artery, although it inhibited the ex vivo platelet aggregation induced by collagen in whole blood to much the same extent as desethyl KBT-3022. Some previous investigators have also found aspirin to be ineffective in a rat model in which arterial thrombosis is induced by either electrical stimulation or an arteriovenous shunt (Ashida et al., 1980; Massad et al., 1987). Although the reason for this discrepancy is not entirely clear, it may result from interference by the metabolite of aspirin, salicylate (Merino et al., 1980), or from inhibition of tissue-derived prostacyclin which exerts an antithrombotic effect (Kanayama et al., 1990). In addition, Rao (1987) has reported that aspirin increases platelet spreading across the vascular endothelium.

On the other hand, in the present experiments BM-13505 inhibited both thrombus formation and platelet aggregation, but did not prevent the decline in blood flow or the accompanying cyclic variations. BM-13505 has been reported to be a high-affinity thromboxane A_2 receptor antagonist which has an exceptionally long-lasted action on human platelets (Patscheke et al., 1985) and to completely prevent sudden death produced by the injection of a thromboxane A_2 mimic (U 46619) in rats (Smith and McDonald, 1988). These results are consistent with our previous findings that another thromboxane A_2 receptor antagonist, vapiprost, had a potent effect, whereas aspirin was ineffective in our photochemically induced thrombosis model in rats (Matsuno et al., 1992; Takiguchi et al., 1992a,b; Higo and Karasawa, 1993; Hirata et al., 1993; Schumacher et al., 1993a,b; White et al., 1994). This efficacy of thromboxane A_2 receptor antagonists in our model suggests that thromboxane A_2 receptor activation plays a part in the mechanism underlying arterial thrombosis.

Pretreatment with desethyl KBT-3022 dose-dependently prolonged the time to occlusion of the femoral artery without affecting haemodynamic parameters such as arterial blood pressure and pulse rate. At the same doses, it also inhibited the ex vivo platelet aggregation induced by collagen. In all 6 rats used, particularly at the highest dose (1 mg/kg, i.v.) tested, complete cessation of blood flow could not be obtained during the 30-min observation time following initiation of the photochemical reaction. Furthermore, cyclic variations in blood flow were rarely, if ever, observed in any of the 6 rats, even at the highest dose of desethyl KBT-3022 (1 mg/kg, i.v.).

Desethyl KBT-3022 is the main active metabolite of KBT-3022, which is a potent cyclooxygenase inhibitor. It prevents platelet activation by inhibiting not only cyclooxygenase, but also phospholipase C and/or phospholipase A_2, resulting in suppression of phosphatidic acid and arachidonic acid release from thrombin-stimulated guinea-pig platelets (Yamashita et al., 1990). After oral administration, KBT-3022 is readily metabolized in blood to desethyl KBT-3022, and inhibits the cyclooxygenase in platelets more potently than that in endothelial cells or vascular smooth muscle cells (Yamashita et al., 1990). Collectively, these findings suggest that the antithrombotic effect of desethyl KBT-3022 in our photochemically induced thrombosis model in rats may result from a greater inhibition of cyclooxygenase in platelets than in endothelial cells or vascular smooth muscle cells, and/or additional effects such as inhibition of phospholipase C and/or phospholipase A_2.

In this study on the rat, desethyl KBT-3022 inhibited thrombin-induced aggregation of washed platelets, though such aggregation was not inhibited by aspirin or BM-13505 even at 500 μM. Yamashita et al. (1990) have already shown that, in the guinea pig, desethyl KBT-3022, but not aspirin, inhibits thrombin-induced aggregation in washed platelets. Furthermore, it has been reported that cyclooxygenase inhibitors and thromboxane A_2 receptor antagonists are unable to inhibit thrombin-induced platelet aggregation (Takahara et al., 1990; Savi et al., 1994), which is associated with the phospholipase C pathway (Durante et al., 1992; Vickers, 1993). Accordingly, the inhibitory effect of desethyl KBT-3022 on thrombin-induced platelet aggregation may be due to inhibition of phospholipase C. This result strongly supports our supposition that a mode

of action other than cyclooxygenase inhibition participates in the potent antithrombotic effects of desethyl KBT-3022. Umemura et al. (1995) have shown that a specific thrombin inhibitor, argatroban, delays the time required for thrombotic occlusion of the middle cerebral artery in the photochemically induced thrombosis model in rats. A number of studies of animal models of thrombosis have also indicated that thrombin has an important role in platelet-dependent arterial thrombus formation (Edit et al., 1989; Heras et al., 1990; Jang et al., 1990). Interestingly, it has been reported that the combination of a thrombin inhibitor and a thromboxane A_2 receptor antagonist has a potent antithrombotic effect without cyclic variations in blood flow (Fitzgerald and Fitzgerald, 1989; White et al., 1994). Therefore, the potent antithrombotic effect of desethyl KBT-3022 may be due to its possession of a dual action, via inhibition of both cyclooxygenase and phospholipase C.

Gryglewski et al. (1996) have recently reported that ticlopidine shows not only an immediate antithrombotic action but also thrombolytic actions in humans and cats. We have insufficient data concerning thrombolytic activities of desethyl KBT-3022. It seems, however, that desethyl KBT-3022 does not possess thrombolytic properties, because it has little effect to prevent venous thrombosis, in which ticlopidine is effective (Yokota et al., 1995). On the other hand, we have previously reported that ticlopidine inhibits thrombus formation in our photochemically induced thrombosis model in rats, but does not prevent the decline in blood flow or the accompanying cyclic variations (Takiguchi et al., 1992b). The antithrombotic effects of desethyl KBT-3022 are apparently different from those of ticlopidine, because desethyl KBT-3022 prevents the decline in blood flow or the accompanying cyclic variations. In the light of these experimental facts, it seems that the potent antithrombotic effects of desethyl KBT-3022 in the photochemically induced thrombosis model in rats are not associated with its thrombolytic properties.

Platelet adhesion onto the exposed subendothelium is considered to be a factor initiating thrombus formation. Indeed, aurintricarboxylic acid, which inhibits the interaction between von Willebrand factor and platelet glycoprotein Ib, has been reported to completely inhibit thrombus formation in rat and canine models of thrombosis (Kawasaki et al., 1994; Takiguchi et al., 1996). However, the antithrombotic activity of desethyl KBT-3022 is unlikely to be due to any suppression of platelet adhesion to the exposed subendothelium of the injured artery, because desethyl KBT-3022 (and aspirin) failed to inhibit platelet retention in a collagen-coated-bead column.

In conclusion, our results show that desethyl KBT-3022 potently inhibits thrombus formation without causing cyclic variations in blood flow in the photochemically induced thrombosis model in rats at doses that inhibit the ex vivo platelet aggregation induced by collagen in whole blood. The antithrombotic effect may, in part, result from inhibitory effects on thrombin-induced platelet aggregation exerted via inhibition of phospholipase C and/or phospholipase A_2, in addition to inhibition of cyclooxygenase in platelets. Accordingly, desethyl KBT-3022 should be considered to be of potential therapeutic value in chronic arterial occlusive diseases.

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References

Ashida, S., Sakum, K., Abiko, Y., 1980. Antithrombotic effects of ticlopidine, acetylsalicylic acid and dipyridamole in vascular shunt model in rats. Thromb. Res. 17, 663–671.

Born, G.V.R., Cross, M.J., 1963. The aggregation of blood platelets. J. Physiol. 168, 178–195.

Durante, W., Kroll, M.H., Vanhoutte, P.M., Schafer, A.I., 1992. Endothelium-derived relaxing factor inhibits thrombin-induced platelet aggregation by inhibiting platelet phospholipase C. Blood 76, 110–116.

Edit, J.F., Allison, P., Norle, S., Ashton, J., Golino, P., Mcnatt, J., Buja, L.M., Willerson, J.T., 1989. Thrombin is an important mediator of platelet activation in stenosed canine coronary arteries with endothelial injury. J. Clin. Invest. 84, 18–27.

Fitzgerald, D.J., Fitzgerald, G.A., 1989. Role of thrombin and thromboxane A_2 in reocclusion following coronary thrombolysis with tissue-type plasminogen activator. Proc. Natl. Acad. Sci. USA 86, 7585–7589.

Gryglewski, R.J., Korbut, R., Swies, J., Kostka-Trabka, E., Bieron, K., Robak, J., 1996. Thrombolytic action of ticlopidine: possible mechanisms. Eur. J. Pharmacol. 308, 61–67.

Heras, M., Chesebro, J.H., Webster, M.W.I., Mruk, J.S., Grill, D.E., Penny, W.J., Bowie, E.J.W., Badimon, L., Fuster, V., 1990. Hirudin, heparin, and placebo during deep arterial injury in the pig: the in vitro role of thrombin in platelet-mediated thrombosis. Circulation 82, 1476–1484.

Higo, K., Karasawa, A., 1993. Inhibitory effect of KW-3635, a new thromboxane A_2-receptor antagonist, on arterial thrombosis in guinea pigs. Jpn. J. Pharmacol. 63, 521–523.

Hirata, Y., Takiguchi, Y., Wada, K., Matsuno, H., Umemura, K., Uematsu, T., Nakashima, M., 1993. Roles of platelet-activation factor, thromboxane A_2, ADP and thrombin in thrombogenesis in the guinea pig. Eur. J. Pharmacol. 231, 421–425.

Jang, I.K., Gold, H.K., Ziskind, A.A., Leinbach, R.C., Fallon, J.T., Collen, D., 1990. Prevention of platelet-rich arterial thrombosis by selective thrombin inhibitor. Circulation 81, 219–225.

Kawasaki, T., Kaku, S., Kohinata, T., Sakai, Y., Taniuchi, Y., Kawamura, K., Yano, S., Takenaka, T., Fujimura, Y., 1994. Inhibition by aurintricarboxylic acid of von Willebrand factor binding to platelet GPIb, platelet retention, and thrombus formation in vivo. Am. J. Hematol. 47, 6–15.

Kanayama, T., Kimura, Y., Iseki, K., Hayashi, Y., Tamao, Y., Mizogami, S., 1990. Antithrombotic effects of KP-10614, a novel and stable prostacyclin (PGI_2) analog. J. Pharmacol. Exp. Ther. 255, 1210–1217.

Massad, L., Plotkine, M., Capdeville, C., Boulu, R.G., 1987. Electrically induced arterial thrombosis model in the conscious rat. Thromb. Res. 48, 1–10.

Matsuno, H., Umematsu, T., Umemura, K., Takiguchi, Y., Wada, K., Nakashima, M., 1992. Effects of vapiprost, a novel thromboxane receptor antagonist, on thrombus formation and vascular patency after thrombolysis by tissue-type plasminogen activator. Br. J. Pharmacol. 106, 533–538.

Merino, J., Livio, M., Rajtar, G., De Gaetano, G., 1980. Salicylate reverses in vitro aspirin inhibition of rat platelet and vascular prostaglandin generation. Biochem. Pharmacol. 29, 1093–1096.

Patscheke, H., Hornberger, W., Stegmeier, K., 1985. Blockade of the human platelet thromboxane A_2 receptor by 4-(2-(*p*-chlorobenzene)sulphonylamino)ethylbenzene acetic acid (BM-13505). In: Int. Symp. Leukotrienes Prostanoids Hlth. Dis., Tel Aviv. p. 11.

Rao, G.H.R., 1987. Influence of anti-platelet drugs on platelet-vessel wall interactions. Prostaglandins Leukotrienes Med. 30, 133–145.

Roth, G.J., Calverley, D.C., 1994. Aspirin, platelets, and thrombosis: theory and practice. Blood 83, 885–898.

Savi, P., Bernat, A., Dumas, A., Aït-Chek, L., Herbert, J.-M., 1994. Effect of aspirin and clopidogrel on platelet-dependent tissue factor expression in endothelial cells. Thromb. Res. 73, 117–124.

Schumacher, W.A., Heran, C.L., Youssef, S., Megill, J.R., Michel, I., Durham, S.K., 1993a. Comparison of a thromboxane receptor antagonist and aspirin in experimental arterial thrombosis. Haemostasis 23, 219–228.

Schumacher, W.A., Steinbacher, T.E., Heran, C.L., Megill, J.R., Durham, S.K., 1993b. Effects of antithrombotic drugs in a rat model of aspirin-insensitive arterial thrombosis. Thromb. Haemost. 69, 509–514.

Smith, E.F., McDonald, J., 1988. Protective effects of the thromboxane receptor antagonists BM 13.177 and BM 13.505 against U 46619-induced sudden death in rats. Pharmacology 36, 340–347.

Takahara, K., Murray, R., Fitzgerald, G.A., Fitzgerald, D.J., 1990. The response to thromboxane A_2 analogues in human platelets. J. Biol. Chem. 265, 6836–6844.

Takiguchi, Y., Hirata, Y., Wada, K., Nakashima, M., 1992a. Arterial thrombosis model with photochemical reaction in guinea-pig and its property. Thromb. Res. 67, 435–445.

Takiguchi, Y., Wada, K., Nakashima, M., 1992b. Comparison of the inhibitory effects of the TXA_2 receptor antagonist, vapiprost, and other antiplatelet drugs on arterial thrombosis in rats: possible role of TXA_2. Thromb. Haemost. 68, 460–463.

Takiguchi, Y., Shimazawa, M., Nakashima, M., 1996. A comparative study of the antithrombotic effect of aurintricarboxylic acid on arterial thrombosis in rats and guinea-pigs. Br. J. Pharmacol. 118, 1633–1638.

Umemura, K., Kawai, H., Ishihara, H., Nakashima, M., 1995. Inhibitory effect of clopidogrel, vapiprost and argatroban on the middle cerebral artery thrombosis in the rat. Jpn. J. Pharmacol. 67, 253–258.

Vickers, J.D., 1993. U73122 affects the equilibria between the phosphoinositides as well as phospholipase C activity in unstimulated and thrombin-stimulated human and rabbit platelets. J. Pharmacol. Exp. Ther. 266, 1156–1163.

White, B.P., Sullivan, A.T., Lumley, P., 1994. Prevention of intracoronary thrombosis in the anaesthetised dog: the importance of thromboxane A_2 and thrombin. Thromb. Haemost. 71, 366–374.

Yamashita, A., Matsuo, K., Yokota, K., Ito, K., Nurimoto, S., 1990. Anti-platelet effect and mode of action of a new anti-platelet agent KBT-3022. Eur. J. Pharmacol. 183, 340.

Yokota, K., Yamashita, A., Oda, M., 1995. Anti-thrombotic activity of KBT-3022 in experimental models of thrombosis. Jpn. J. Pharmacol. 68, 201–206.