Inhibitory Effect of KW-3635, a New Thromboxane A_2-Receptor Antagonist, on Arterial Thrombosis in Guinea Pigs

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ABSTRACT—The antithrombotic effects of the thromboxane (TX) A_2-receptor antagonist and aspirin were determined using a photochemically-induced arterial thrombosis model in the femoral arteries of guinea pigs. KW-3635 (sodium (*E*)-11-[2-(5,6-dimethyl-1-benzimidazolyl)ethylidene]-6,11-dihydrodibenz[*b,e*]-oxepine-2-carboxylate monohydrate) and BM-13505, both of which are TXA_2-receptor antagonists, and aspirin inhibited the thrombus formation at the doses that inhibited the ex vivo platelet aggregation induced by sodium arachidonate (100 μM) or collagen (3 μg/ml). These results support the notion that TXA_2 is an important mediator in the present model of arterial thrombogenesis.

Keywords: KW-3635, Thromboxane A_2-receptor antagonist

Several experimental models of arterial thrombosis associated with endothelial injury have been established. The way of inducing endothelial injury varies among the models, and endothelial injury has been induced mechanically, chemically or electrically and so on. Injured endothelial cells become unable to release antithrombotic substances such as prostaglandin I_2 (PGI_2) or endothelial derived relaxing factor. In parallel to this, thrombogenic substances like thromboxane (TX) A_2 or 5-hydroxytryptamine become dominant, which entails arterial thrombosis. Matsuno et al. (1) recently modified a model of arterial thrombosis based on a photochemically-induced reaction (2). The thrombus produced in this model is characterized by its close resemblance to the one that forms intravascularly in peripheral vascular disease clinically (1).

The platelets from guinea pigs are quite similar to those from humans with regard to their response to agonists and antagonists of the prostaglandins (3). Therefore, there is fairly general agreement that the guinea pig is a suitable species for evaluating the antithrombotic effect of an agent. Using guinea pigs, several antithrombotic and antiplatelet agents were demonstrated to exhibit preventive effects on thrombus formation. These agents include TXA_2-receptor antagonists (4, 5), a TXA synthetase inhibitor (5), aspirin (4) and ticlopidine (5).

KW-3635 (sodium (*E*)-11-[2-(5,6-dimethyl-1-benzimidazolyl)ethylidene]-6,11-dihydrodibenz[*b,e*]oxepine-2-carboxylate monohydrate) is a potent and selective TXA_2-receptor antagonist, which has been demonstrated to inhibit the binding of a TXA_2 agonist and a TXA_2 antagonist to its receptors (6), TXA_2-mediated platelet aggregation (7) and constriction of various smooth muscle preparations in response to TXA_2 (8). Moreover, KW-3635 exhibits antithrombotic activity as evidenced by its preventive effect on vascular reocclusion after thrombolysis with tissue-type plasminogen activator (9). The purpose of the present study was to determine whether the TXA_2-receptor antagonist KW-3635 is effective in this photochemically-induced thrombosis model and to compare its efficacy with those of BM-13505 (4-[2-(4-chlorobenzenesulfonamide)-ethyl]-benzene acetic acid) (7, 8) and aspirin. BM-13505 is a non-prostanoid type TXA_2-receptor antagonist that has nearly the same potency as KW-3635 in guinea pigs. We also examined the relationship between the antithrombotic and antiplatelet potency of these agents.

KW-3635 and BM-13505 were synthesized in our laboratories. Sodium arachidonate and rose bengal were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Collagen and aspirin were obtained from Hormon-Chemie (München, Germany) and Nacalai Tesque, Inc. (Kyoto), respectively. All the drugs were suspended in 0.3% sodium carboxymethylcellulose at an appropriate concentration so as to make its administration volume 5 ml suspension per kg body weight. All the drugs were administered orally 2 hr before the experiment.

Male Hartley guinea pigs weighing 280–400 g (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu) were used. Arterial thrombosis was produced with a slight modification of the method of Takiguchi et al. (4). In brief, a 5-mm segment of the right femoral artery distal to the inguinal ligament was separated carefully, and a pulse doppler flow probe (PDV-20; Crystal Biotech America, Hopkinton, MA, USA) was placed for measuring blood flow. The arterial blood flow was continuously monitored on a pen-recorder (WI-641GC; Nihon Kohden, Tokyo). The irradiation of green light (540 nm, 180,000 lux) (L4887; Hamamatsu Photonics, Hamamatsu) on the right femoral artery proximal to the flow probe was started, and then after 10 min, 3 mg/kg (i.v.) rose bengal was infused for 3 min. Green light exposure was continued until 20 min after the end of rose bengal infusion. The time to complete occlusion was recorded, and the occlusion time of the non-occluded animal was regarded as 20 min. For the analysis of the time to occlusion, the Kruskal-Wallis test followed by the Steel multiple comparison test was used. For the analysis of the incidence of occlusion, Fisher's exact probability test was used. P values less than 0.05 were considered to be statistically significant.

Antithrombotic effects of the test compounds are shown in Table 1. In the vehicle-treated group, the blood flow of the irradiated artery was completely diminished within 400 sec, and it did not recover until 1200 sec. In contrast, all three of the compounds tested prolonged the time to occlusion. KW-3635 and BM-13505, both of which are TXA_2-receptor antagonists, delayed the thrombus formation at the dose of 10 mg/kg. Aspirin at the doses of 10 and 100 mg/kg prolonged the time to occlusion.

In another series of experiments, ex vivo platelet aggregation was determined. Blood was withdrawn from the abdominal aorta and mixed with 1/10 volume of 3.8% sodium citrate. Platelet aggregation was determined by measuring the optical density of platelet-rich plasma using an aggregometer (TE500; Erma Optical Works Co., Ltd., Tokyo). For the analysis of ex vivo platelet aggregation, the Kruskal-Wallis test followed by a Tukey-type multiple comparison test was used.

Figure 1 shows the effects of the test compounds on ex vivo platelet aggregation induced by sodium arachidonate (100 μM) and collagen (3 μg/ml). Sodium arachidonate is converted by cyclooxygenase and TXA synthetase to TXA_2, which induces platelet aggregation. In guinea pigs,

Table 1. Frequency of femoral artery occlusion in the photochemically-induced thrombosis model in guinea pigs

Groups		n	Occlusion[a] incidence	Occlusion[b] time (sec)
Vehicle		8	8/8	383± 40
KW-3635	3 mg/kg	8	6/8	661±127
	10	8	2/8**	1040±107**
	30	8	0/8***	1200 **
BM-13505	3 mg/kg	8	5/8	693±162
	10	8	2/8**	1052±110*
Aspirin	3 mg/kg	8	6/8	654±134
	10	8	4/8*	992± 94**
	100	8	4/8*	977±118*

***P<0.001, **P<0.01, *P<0.05: significantly different from the vehicle-treated group by Fisher's exact probability test[a] and by the Steel multiple comparison test[b]. Animals in all the treated groups were administered a drug orally at 2 hr before the experiment. Non-occluded arteries were defined as those that did not show complete occlusion within 20 min. The occlusion time of a non-occluded artery was regarded as 20 min (1200 sec).

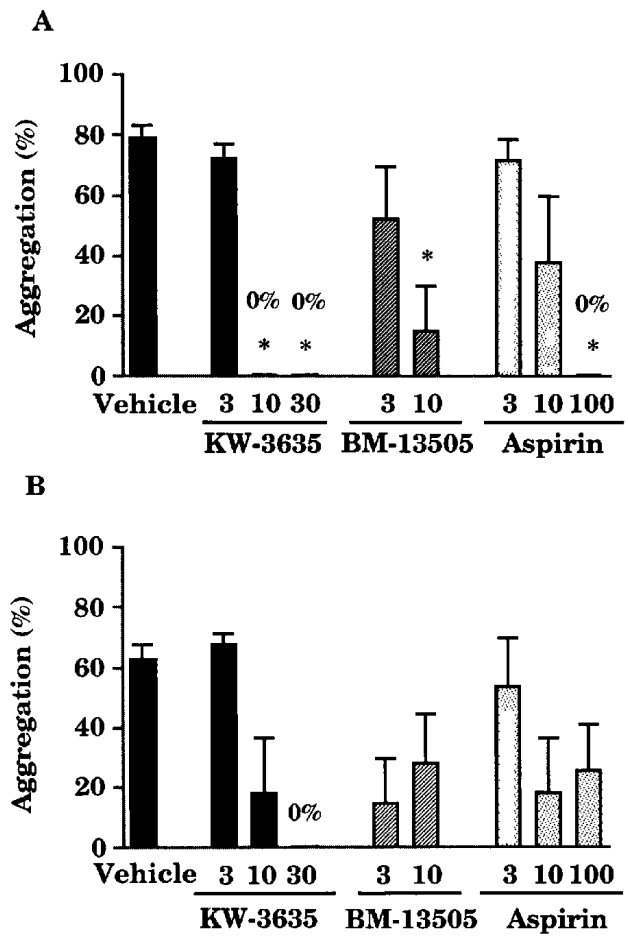

Fig. 1. Inhibitory effects of KW-3635, BM-13505 and aspirin on ex vivo platelet aggregation induced by sodium arachidonate (100 μM) (A) and collagen (3 μg/ml) (B). Each drug was orally administered 2 hr before the experiment. Each bar indicates the mean±S.E. of 4 experiments. *P<0.05, significantly different from the vehicle-treated group by the non-parametric Tukey-type multiple comparison test.

the aggregatory response to collagen is mainly mediated by TXA_2 (10). All the drugs almost completely suppressed the aggregation induced by sodium arachidonate at the each maximum dose used. Collagen-induced platelet aggregation was also markedly attenuated by pre-treatment with these drugs.

The minimum effective doses of KW-3635 and BM-13505 to prolong the time to occlusion were 10 mg/kg for both the drugs. These doses corresponded to the minimum effective doses to inhibit the ex vivo platelet aggregation induced by sodium arachidonate, suggesting that the antithrombotic effects of these compounds are dependent on their abilities to inhibit TXA_2-mediated platelet aggregation. A high dose of KW-3635 (30 mg/kg) completely abolished collagen-induced platelet aggregation, which may suggest a possible additional unknown mechanism for this agent in inhibiting platelet aggregation. Treatment with aspirin also prolonged the time to thrombotic occlusion at the doses inhibiting the ex vivo platelet aggregation by sodium arachidonate or collagen. These results suggest that the compounds that inhibit TXA_2-mediated platelet aggregation could be effective against arterial thrombogenesis.

In the former studies, Takiguchi et al. (4) and Hirata et al. (5) suggested that in guinea pigs, TXA_2 and ADP are the main components involved in producing the photochemically-induced thrombus. The present result is in accordance with their notion that TXA_2 is one of the most important mediators in thrombogenesis in guinea pigs.

In summary, the present study demonstrated that KW-3635, a TXA_2-receptor antagonist, is effective in the guinea pig model of photochemically-induced thrombosis. The antithrombotic potency of the agent correlated well with its potency to inhibit TXA_2-mediated platelet aggregation. These results support the notion that TXA_2 is an important mediator in the present model of arterial thrombogenesis.

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