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Effect of KBT-3022, a New Diphenylthiazole Derivative, on Platelet Functions

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Abstract

The effects of KBT-3022 and its metabolite desethyl KBT-3022 on platelet aggregation were determined in rat, guinea-pig, rabbit and human platelets in-vitro and ex-vivo.

KBT-3022 and desethyl KBT-3022 inhibited platelet aggregation induced by arachidonic acid and collagen in-vitro more potently than aggregation induced by adenosine diphosphate, platelet-activating factor or thrombin, as well as by acetylsalicylic acid, and their effects were approximately 100 times more potent than those of acetylsalicylic acid.

Desethyl KBT-3022, but not KBT-3022 or acetylsalicylic acid, inhibited thrombin-induced aggregation and 5-hydroxytryptamine release from platelets more potently than ticlopidine hydrochloride at higher concentrations. Oral administration of KBT-3022 inhibited both arachidonic acid- and collagen-induced platelet aggregation and reduced platelet retention in a glass-bead column approx. 100 times more potently than acetylsalicylic acid. KBT-3022 showed little or no anti-inflammatory effect on either ultraviolet-induced erythema or arachidonic acid induced ear oedema, and had lower gastro-ulcerogenicity than acetylsalicylic acid.

These results suggest that KBT-3022 is a potent inhibitor of platelet activation with weak side-effects.

It is well recognized that platelet adhesion, platelet aggregation and the concomitant release of platelet constituents play an important role in the pathogenesis of occlusive vascular disease and atherosclerosis (Mustard & Packham 1970; Ross & Glomset 1976 a, b). Many platelet aggregation inhibitors have been subjected to clinical trials in patients with these diseases. So far, one of these drugs, acetylsalicylic acid (ASA), has been proved to be effective in many clinical trials for prevention of transient ischaemic attack, stroke and myocardial infarction (ATC 1994; ISIS-2 1988). However, the long-term use of ASA is associated with adverse side effects, including gastric mucosal injury (AMIS 1980; Bousser et al 1983). Thus, it is necessary to develop a more potent platelet-aggregation inhibitor producing little or no injury to the gastric mucosa.

KBT-3022, ethyl 2-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]pyrrol-1- ylacetate, is a chemically novel platelet-aggregation inhibitor (Seko et al 1989) synthesized at Kanebo (Fig. 1). This paper describes the effects of KBT-3022 on platelet aggregation and 5-HT release from platelets in-vitro, and its effects on platelet aggregation and platelet adhesion ex-vivo, in comparison with those of ASA (Vane 1994) and ticlopidine hydrochloride (Ashida & Abiko 1978), which is a well known platelet aggregation inhibitor.

Moreover, the anti-inflammatory activity and gastro-ulcerogenicity of KBT-3022 were investigated in comparison with those of ASA and/or ticlopidine. KBT-3022 has been shown to be metabolized rapidly to desethyl KBT-3022 (Fig. 1), and to be undetectable in blood of mice, rats or dogs after oral administration (Nakada et al 1990; Nakada et al 1993). Therefore, we also examined the effects of this metabolite in-vitro.

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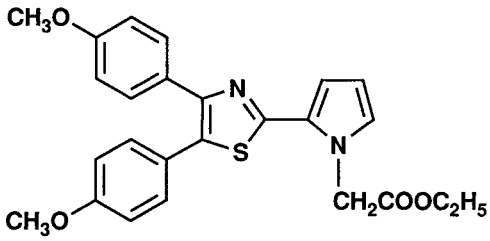
Materials and Methods

Animals

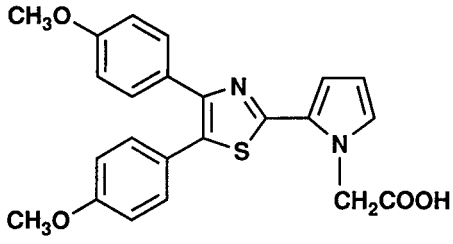
Mice (ddY strain, 21-31 g), rats (Wistar strain, 200-290 g), guinea-pigs (Hartley strain, 210-610 g) and Japanese albino rabbits (2·5–3·5 kg) were used. All of the animals were males and had been acclimatized for at least several days before drug administration. Reagents KBT-3022 and desethyl KBT-3022 were synthesized, and ticlopidine was extracted from Panaldine tablets purchased from Daiichi Pharmaceutical (Tokyo, Japan) and purified at the New Drug Research Laboratories of Kanebo, Ltd. The chemical purity was confirmed by elemental analysis, the error for each of carbon, hydrogen and nitrogen being less than 0·3%. ASA was purchased from Wako Pure Chemical Industries (Osaka, Japan). These drugs were dissolved in dimethylsulphoxide (DMSO) for use in in-vitro experiments. For oral administration, the drugs were dissolved or suspended in 0·5% polyoxyethylene sorbitan mono-oleate solution. Arachidonic acid sodium salt and free acid and adenosine 5-diphosphate sodium salt (ADP) from Sigma Chemical Co. (St Louis, MO, USA), platelet-activating factor (1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine, PAF) from Calbiochem (San Diego, CA, USA), collagen from Hormon-Chemie (Munich, Germany) and thrombin from Mochida Pharmaceutical (Tokyo, Japan) were used.

Preparation of platelet-rich plasma (PRP)

Blood from guinea-pigs, rabbits and healthy male human volunteers was drawn into test tubes containing a one-tenth



KBT-3022



Desethyl KBT-3022

FIG. 1. Chemical structures of KBT-3022 (ethyl 2-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]pyrrol-1-ylacetate) and its metabolite desethyl KBT-3022.

volume of 3·8% trisodium citrate. Blood from rats was drawn into test tubes containing a one-tenth volume of 2·8% trisodium citrate. PRP and platelet-poor plasma (PPP) were prepared from citrated blood by centrifugation. For aggregation studies, platelet counts in PRP from rats and guinea-pigs were adjusted to 5×10^8 mL^{-1}, that from rabbits to $2–5 \times 10^8$ mL^{-1} and that from man to 3×10^8 mL^{-1}, with PPP.

Preparation of washed platelets

Washed platelets were prepared by the method described by Ushikubi et al (1987). A one-tenth volume of 77 mM sodium EDTA, pH 7·4, was added to PRP, and the platelets were separated by centrifugation at 400 *g* for 10 min. The platelets were washed once and resuspended in assay buffer (10 mM HEPES, 145 mM NaCl, 5 mM KCl, 0·5 mM Na_2HPO_4 and 6 mM glucose, pH 7·4). Calcium chloride was added to the suspension at 1 mM before the thrombin-induced aggregation or secretion study.

Platelet aggregation in-vitro

Determination of platelet aggregation was performed by the method of Born (1962) using a Platelet Aggregation Profiler (PAP-3, Bio/Data, Horsham, PA, USA) or NKK Hematracer (PAT-4A, Niko Bioscience, Tokyo, Japan). PRP containing a 1/100 volume of test drug or DMSO was incubated for 5 min at 37°C. The maximum increase in light transmission determined from the aggregation curve for 5 min was defined as the percentage of aggregation. Percentage inhibition of aggregation was calculated as:

$$\text{inhibition \%} = (1 - \text{\% aggregation (drug)}/\text{\%aggregation (vehicle)}) \times 100 \quad (1)$$

[^{3}H]5-HT release in-vitro

Guinea-pig PRP was incubated for 1 h at room temperature with 1·11 MBq [^{3}H]5-HT (5-hydroxy[G-^{3}H]tryptamine creatinine sulphate, 1·55 GBq mg^{-1}, Amersham International plc, Amersham, UK). The platelets were separated by centrifugation and resuspended in PPP or the assay buffer. Platelet suspension (365 μL) was incubated with 4 μL of a test drug or DMSO for 5 min at 37°C. Forty microlitres of 200 μg mL^{-1} collagen, 1 unit mL thrombin or 25 mM Tris-HCl-buffered saline pH 7·4 (as a blank) was added to the suspension, and the reaction mixture was agitated. Five minutes later, 400 μL ice-cold 3% formaldehyde was added to the platelet suspension to stop the release reaction (Costa & Murphy 1975). Samples were centrifuged at 10 000 *g* at 4°C for 2 min, and 400 μL supernatant was removed for radioactivity counting. Instead of 3% formaldehyde, 2% Triton X-100 was added to determine the total radioactivity. The percentage of [^{3}H]5-HT release from platelets was calculated as:

$$\text{release \%} = (A - C)/(B - C) \times 100 \quad (2)$$

where A is the radioactivity of agonist added, B is the total radioactivity and C is the radioactivity of the blank. Percentage inhibition of release was calculated as:

$$\text{inhibition \%} = (1 - \text{release \% (drug)}/\text{release \% (vehicle)}) \times 100 \quad (3)$$

Platelet aggregation ex-vivo

Fasted guinea-pigs were given a test drug or vehicle orally. At the scheduled time after administration of the drug, blood was collected from the abdominal aorta under ether anaesthesia. Platelet aggregation was measured by the same method as that for the in-vitro experiment. The aggregating agents used were 0·1 mM arachidonic acid or 10 μg mL^{-1} collagen. The inhibitory effects of the drugs were determined by calculating the percentage inhibition with respect to vehicle-treated animals.

Platelet retention

Fasted guinea-pigs were administered test drugs or the vehicle orally. At 3 h after drug administration, each guinea-pig was anaesthetized with sodium pentobarbitone (20 mg kg^{-1}, i.p.), and a mid-line abdominal incision was made. A polyethylene cannula was inserted into the abdominal aorta and connected to a column of glass beads (Igakushoin Inc., Tokyo, Japan), which had been connected to a plastic syringe containing 100 μL 2% EDTA-2K saline placed on an infusion pump. One millilitre of blood was withdrawn for a determination of platelet count before passage through the glass bead column, and then the same volume of blood was withdrawn through the column at a constant flow rate of 4 mL min^{-1} into the plastic syringe. Platelet counts were determined with a Coulter Counter. The percentage retention of platelet was calculated as:

$$\text{retention \%} = (A - B)/A \times 100 \quad (4)$$

where A is the platelet count before passing through the glass bead column, and B is the platelet count after passing through the glass bead column.

Table 1. Effects of KBT-3022, desethyl KBT-3022, ASA and ticlopidine on platelet aggregation induced by various agents in several species including man.

	IC50 (μM)[a] KBT-3022	Des-KBT[b]	ASA	Ticlopidine
Man				
collagen (2 μg mL^{-1})	0·22	0·20	28	400
Rabbit				
arachidonic acid (0·5 mM)	1·5	0·39	27	750
collagen (20 μg mL^{-1})	0·43	0·36	35	450
Guinea-pig				
arachidonic acid (0·1 mM)	0·45	0·37	59	260
collagen (10 μg mL^{-1})	0·15	0·24	34	430
ADP (2 μM)	> 100	> 100	> 1000	310
PAF (5 nM)	> 100	> 100	> 1000	300
thrombin (0·05 units mL^{-1})	> 10	5·0	> 1000	33
Rat				
collagen (30 μg mL^{-1})	0·92	0·58	53	260

[a]IC50 values were calculated by a least-squares linear regression analysis from concentration-response curves of 3–5 experiments, [b]desethyl KBT-3022.

Gastric mucosal lesion

The fasted mice and guinea-pigs were given test drugs orally and killed by ether anaesthesia 6 and 3 h later, respectively. Ten minutes before they were killed, 5% pontamin sky blue 6B (Tokyo Kasei Kogyo Co., Tokyo, Japan) dissolved in saline was injected intravenously. The stomach was removed, inflated and fixed by 1% formalin. The stomach was opened along the greater curvature and the length of lesions in the glandular portion was measured under a dissecting microscope (×10). The incidence of lesions was determined, and the sum of the lengths of all the lesions in each animal was defined as the ulcer index.

Arachidonic acid-induced ear oedema

Arachidonic acid-induced ear oedema in mice was produced using the method described by Young et al (1983). Briefly, fed mice were administered a test drug or vehicle orally. One hour later, 2 mg mL^{-1} arachidonic acid solution was applied in a 10-μL volume to both the inner and outer surfaces of the right ear using a micropipet. One hour later, the thickness of the right and left ears was measured using a caliper gauge. Oedema was determined by subtracting the thickness of the left ear from that of the right.

Ultraviolet-induced erythema

Ultraviolet-induced erythema was produced according to the method described by Winder et al (1958). Briefly, circumscribed areas of skin of guinea-pigs, depilated 18 h earlier, were masked by a glove with three closely placed 9-mm holes, and were subsequently exposed to an ultraviolet-light source for 60 s. Two and 5 h after irradiation, the erythema was scored subjectively by three persons according to the completeness of the circle of erythema. An exposed area which developed no evident erythema was scored as 0, while one with a full circle of definite redness was scored as 1. A spot with partial redness was scored as 0·5. Thus, we counted the total 3-spot scores and summed the total score made by the three observers. Because the scoring was subjective, it was imperative for the assay to be conducted on a blind basis. Drugs were administered orally 1 h before irradiation.

Statistics

Each value was expressed as the mean or the mean ± s.e. Least-squares linear regression analysis was used to calculate the IC50 or ED50 value. The statistical significance of the data was evaluated using one way analysis of variance or the Kruskal-Wallis test, followed by Dunnett's test. Differences with P values of less than 0·05 were considered statistically significant.

Results

Platelet aggregation in-vitro

KBT-3022, desethyl KBT-3022, ASA and ticlopidine inhibited collagen-induced aggregation in human PRP in a concentration-dependent manner. The IC50 values for these drugs in platelet aggregation induced by various agents are summarized in Table 1. The inhibitory effects of KBT-3022 and desethyl KBT-3022 on arachidonic acid- or collagen-induced aggregation were approximately equal and approximately 100 and 1000 times as potent as those of ASA and ticlopidine, respectively. KBT-3022 and ASA inhibited the aggregation induced by arachidonic acid and collagen, but not that by ADP, PAF or thrombin up to the maximal concentration which was dissolved in the medium used. Desethyl KBT-3022 inhibited not only arachidonic acid- and collagen-induced aggregation but also thrombin-induced aggregation at an approximately 10 times higher concentration.

The respective inhibitory effects of KBT-3022, desethyl KBT-3022, ASA and ticlopidine on collagen-induced aggregation of PRP from rats, guinea-pigs and rabbits were the same as those on that of human PRP.

[^{3}H]5-HT release from platelets in-vitro

The mean release of [^{3}H]5-HT-labelled guinea-pig platelets induced by collagen and thrombin were 44·0% and 76·1%, respectively, and the radioactivity of the blank was below 20% of the total. As shown in Fig. 2, all of the drugs tested inhibited collagen-stimulated 5-HT release from platelets in a concentration-dependent manner, yielding IC50 values of 0·27 μM for KBT-3022, 0·36 μM for desethyl KBT-3022,

Table 2. Effect of KBT-3022, ASA and ticlopidine on platelet retention in a column of glass beads.

Drug	Dose (mg kg^{-1}, p.o.)	n	Platelet retention (%)	Inhibition (%)
Control		20	71·6 ± 1·0	
KBT-3022	0·1	6	66·1 ± 3·0	7·7
	0·3	6	68·4 ± 3·1	4·5
	1	6	53·4 ± 5·4**	25·3
	3	6	53·8 ± 2·5**	24·9
ASA	30	6	69·0 ± 2·6	3·6
	100	6	58·1 ± 6·9*	18·9
	300	6	47·1 ± 5·8**	34·3
Ticlopidine	300	6	66·3 ± 3·9	7·4

Each data represents the mean ± s.e. *$P < 0.05$, **$P < 0.01$ compared with control (Dunnett's test).

70 μM for ASA and 470 μM for ticlopidine. The thrombin-stimulated release of 5-HT from platelets was not inhibited by KBT-3022 or by ASA. However, desethyl KBT-3022 produced inhibition in a concentration-dependent manner yielding an IC50 value of 7·8 μM. Ticlopidine produced partial inhibition between 100 and 1000 μM.

Platelet aggregation ex-vivo

As shown in Fig. 3, KBT-3022 produced a dose-dependent inhibition of arachidonic acid- and collagen-induced platelet aggregation, yielding IC50 values of 0·066 and 0·21 mg kg^{-1}, respectively 3 h after oral administration. ASA also inhibited the aggregation, yielding IC50 values of 44 and 54 mg kg^{-1}, respectively. However, ticlopidine (300 mg kg^{-1}, p.o.) inhibited collagen-induced aggregation slightly. In order to compare the duration of the inhibitory effect of KBT-3022 with that of ASA on platelet aggregation, the ED50 values for inhibition of arachidonic-induced platelet aggregation were determined at 1, 2, 4, 6, 24 and 72 h after oral administration. As shown in Fig. 4, the inhibitory effect of KBT-3022 reached a maximum at 6 h and an apparent effect was observed even at 72 h after administration. The ED50 value of ASA was constant from 2 to 72 h after administration.

Platelet retention

In guinea-pigs, KBT-3022 (1 and 3 mg kg^{-1}, p.o.) significantly reduced the platelet retention in a glass bead column (Table 2). Similarly, ASA (100 and 300 mg kg^{-1}, p.o.) significantly reduced the retention. However, ticlopidine (300 mg kg^{-1}, p.o.) did not affect the retention. None of these drugs affected the haematocrit values (data not shown).

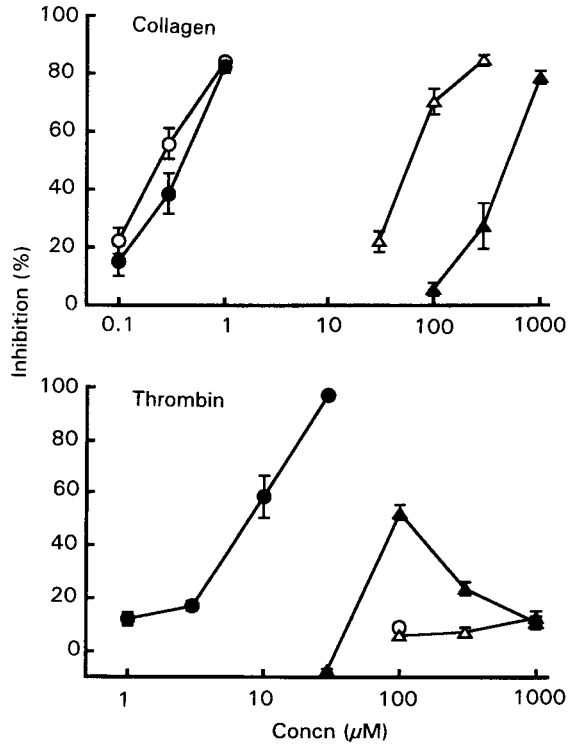


FIG. 2. Effects of KBT-3022 and desethyl KBT-3022 on 5-HT release from guinea-pig platelets stimulated by collagen and thrombin in-vitro. Each point and vertical bar represent the mean and s.e. for 5 experiments, respectively. ○ KBT-3022, ● desethyl KBT-3022, △ ASA, ▲ ticlopidine.

Gastric mucosal lesions

Oral administration of KBT-3022 had no effect on the gastric mucosa at a dose of 30 mg kg^{-1}, but induced a small area of lesions in the corpus mucosa in mice and guinea pigs at 100 mg kg^{-1} (Table 3). In contrast, ASA (10-100 mg kg^{-1}, p.o.) produced lesions in the gastric mucosa dose-dependently.

Table 3. Gastro-ulcerogenic activity of KBT-3022 and ASA.

Drug	Dose (mg kg^{-1}, p.o.)	Ulcerogenicity			
		Mouse incidence	Ulcer index[1]	Guinea-pig incidence	Ulcer index[a]
Control		0/12	0	0/6	0
KBT-3022	10	0/12	0		
	30	0/12	0	0/6	0
	100	5/12	0·2 ± 0·1	1/6	0·5 ± 0·5
ASA	10	3/11	0·1 ± 0·1	2/6	2·5 ± 2·0
	30	8/12	0·5 ± 0·2	4/6	2·8 ± 1·4
	100	11/12	1·2 ± 0·5	6/6	15·5 ± 2·5

[a]The sum of the lengths of all the lesions in each animal (mm).

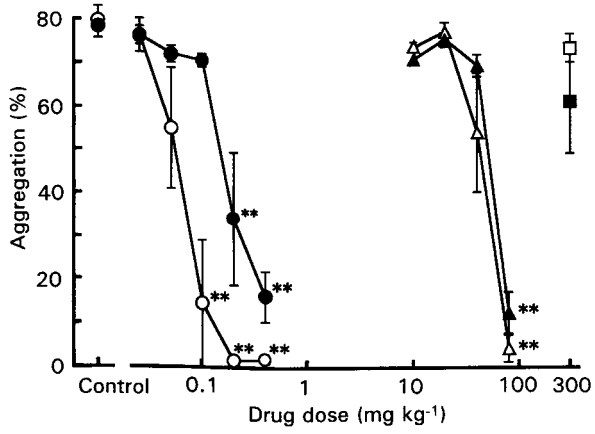


FIG. 3. Effects of oral dose of KBT-3022 on arachidonic acid- and collagen-induced platelet aggregation in guinea-pigs ex-vivo. Each point and vertical bar represent the mean and s.e. for 5 animals, respectively. Clear symbols: arachidonic acid-induced aggregation, solid symbols: collagen-induced aggregation. ○,● KBT-3022, △,▲ ASA, □,■ ticlopidine.

Arachidonic acid-induced ear oedema

The ear oedema induced by topical application of arachidonic acid was not influenced by pretreatment with KBT-3022 (10 and 100 mg kg^{-1}, p.o.) (Table 4). ASA (100 mg kg^{-1}, p.o.) but not ticlopidine (100 mg kg^{-1}, p.o.) inhibited the oedema significantly.

Ultraviolet-induced erythema

KBT-3022 (30 and 100 mg kg^{-1}, p.o.) did not influence the development of erythema at 2 and 5 h after the irradiation in guinea-pigs (Table 5). ASA (30-300 mg kg^{-1}, p.o.) inhibited the development of erythema significantly 2 h after irradiation.

Discussion

KBT-3022 inhibited arachidonic acid- and collagen-induced platelet aggregation but not ADP-, PAF- or thrombin-induced aggregation in-vitro. The inhibitory effects of KBT-3022 on in-vitro aggregation of platelets were equipotent in man, rabbits, guinea-pigs and rats. The profile of the anti-aggregating activity of KBT-3022 was qualitatively similar to that of ASA but not to that of ticlopidine. We have previously reported that KBT-3022 and its metabolite desethyl KBT-3022 inhibit cyclo-oxygenase activity of ovine seminal glands with respective IC50 values of 0·69 and 0·43 μM, but not thromboxane synthetase in platelets (Yamashita et al 1990). As the inhibition of cyclo-oxygenase is produced at the same range of concentration as the inhibition of arachidonic acid- and collagen-induced platelet aggregation, the anti-platelet aggregation activity of KBT-3022 presumably results from inhibition of arachidonic acid metabolism (Silver et al 1973; Vargaftig 1977; Siess et al 1983). The partial inhibition of platelet retention by KBT-3022 and ASA also may result from inhibition of production of thromboxane A_2 or prostaglandin endoperoxides in a glass bead column, which are potent inducers of platelet aggregation (Charo et al 1977).

Huzoor-Akbar & Anwer (1986) proposed that the rat was not an appropriate model with which to study the role of prostaglandins in platelet aggregation. Burke et al (1983) showed that guinea-pig platelets resembled human platelets in their responses to arachidonic acid and 9,11-azo-prostaglandin H_2. Furthermore, the bioavailability of KBT-3022 is low for rats after oral administration (Nakada et al 1993). For these reasons, we used guinea-pigs in order to evaluate the effects of KBT-3022 and other drugs on platelet aggregation.

The inhibitory effect of KBT-3022 on arachidonic acid-

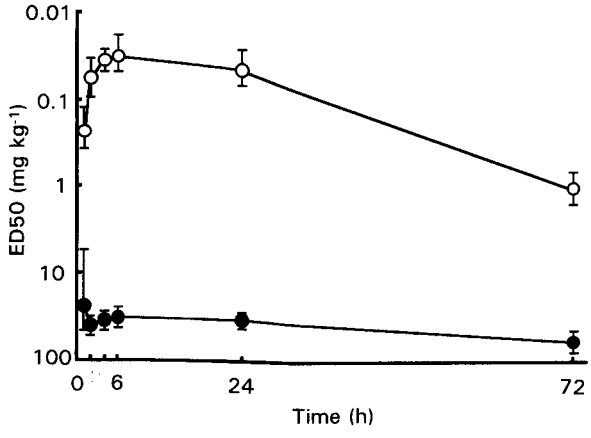


FIG. 4. Time course of the effect of KBT-3022 on arachidonic acid-induced platelet aggregation in guinea-pigs ex-vivo. Each point and vertical bar represent the ED50 for platelet aggregation and its 95% confidence limit, respectively. ○ KBT-3022, ● ASA.

Table 4. Effect of KBT-3022, ASA and ticlopidine on AA-induced ear oedema in mice.

Drug	Dose (mg kg^{-1}, p.o.)	Ear oedema (×100 mm)
Control		24·2 ± 0·6
KBT-3022	10	22·8 ± 0·9
	100	22·6 ± 0·3
ASA	30	21·9 ± 1·8
	100	15·8 ± 1·2**
Ticlopidine	100	21·8 ± 1·7

Each datum point represents the mean ± s.e. for 5 animals. **$P < 0.01$ compared with control (Dunnett's test).

Table 5. Effect of KBT-3022, ASA and ticlopidine on ultraviolet-induced erythema in guinea-pigs.

Drug	Dose (mg kg^{-1}, p.o.)	Score 2 h	5 h[a]
Control		8·3	8·7
KBT-3022	30	7·3	7·0
	100	6·4	6·1
ASA	30	4·3*	6·8
	100	3·8*	6·8
	300	1·4**	5·5**
Ticlopidine	300	7·7	8·8

[a]Time after ultraviolet irradiation. Each datum point represents the mean for 6 animals. *$P < 0.05$, **$P < 0.01$ compared with control (Kruskal-Wallis test followed by Dunnett's test).

induced platelet aggregation in guinea-pigs persisted for 24 h after oral administration and then diminished slowly, whereas the effect of ASA persisted for 72 h. Similar phenomena were also observed in arachidonic acid- and collagen-induced platelet aggregation in rabbits (unpublished data). The time course of anti-aggregation was similar to that of the plasma concentration of desethyl KBT-3022 following oral administration of KBT-3022 to guinea-pigs (unpublished data). These results indicate that the inhibitory effect of KBT-3022 on platelet aggregation is of relatively long duration, but reversible.

A deficiency of endogenous gastric mucosal prostaglandins has been accepted as the major factor in the pathogenesis of gastric lesions caused by cyclooxygenase inhibitors (Whittle et al 1980; Rainsford & Willis 1982). Moreover, these cyclo-oxygenase-derived arachidonic acid metabolites also play an important role in the mediation of acute inflammation such as ultraviolet-induced erythema (Adams et al 1981; Woodward et al 1981) or arachidonic acid-induced ear oedema (Opas et al 1985; Humes et al 1986). KBT- 3022 did not suppress the erythema in guinea-pigs and ear oedema in mice and caused no gastric damage at doses which were sufficient to inhibit platelet aggregation. However, ASA produced anti-inflammatory effects and gastric damage within the same dose range as that reported previously to inhibit platelet aggregation (Kauffman 1989; Vane & Botting 1990). Nakada et al (1994) reported that the radioactivity in the platelet and aorta were 2·4–15·7 times higher and approximately one-half those in plasma, respectively after oral administration of [^{14}C]KBT-3022 to mice. As it has been accepted that the pharmacokinetic behaviour of cyclo-oxygenase inhibitors contributes not only decisively to their therapeutic effects but also to the type and incidence of their side-effects (Pong & Levine 1976; McCormack & Brune 1987), less anti-inflammatory activities and weak gastroulcerogenicity of KBT-3022 may result from its higher concentration in platelets than in other tissues following oral administration.

Qualitatively and quantitatively, desethyl KBT-3022 showed anti-platelet activities similar to those of KBT-3022, except for its effects on platelet activation induced by thrombin in-vitro. Thrombin is known to activate platelets independently of arachidonic acid metabolism (Sano et al 1983; Siess et al 1983). Desethyl KBT-3022 but not KBT-3022 and ASA have been shown to inhibit the release of arachidonic acid from, and the production of phosphatidic acid in thrombin-stimulated platelets, and cAMP phosphodiesterase in platelets (Yamashita et al 1990). Although the concentration of desethyl KBT-3022 required for the inhibition of thrombin-induced platelet activation is approximately 10 times that of arachidonic acid- or collagen-induced platelet activation, it is undeniable that these effects comprise synergistic inhibition of platelet activation. These findings suggest that KBT-3022 may be a potent and long-lasting anti-platelet agent with less side-effects, and as such represents a major therapeutic advance.

Acknowledgments

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A preliminary report of this work has been given at the 61st General Meeting of the Japanese Pharmacological Society, Fukuoka, Japan, in March 1988 (Yokota et al 1988).

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