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European Journal of Pharmacology 439 (2002) 43–52

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# Pharmacological properties of YM-57029, a novel platelet glycoprotein IIb/IIIa antagonist

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Received 8 November 2001; received in revised form 1 February 2002; accepted 19 February 2002

**Abstract**

The pharmacological properties of YM-57029 ({4-[4-(4-carbamimidoylphenyl)-3-oxopiperazin-1-yl]piperidino}acetic acid monohydrochloride trihydrate), a novel glycoprotein IIb/IIIa antagonist were examined in this study. YM-57029 inhibited fibrinogen binding to purified glycoprotein IIb/IIIa, 1000-fold more potently than the tetrapeptide arginine–glycine–aspartic acid–serine (RGDS). YM-57029 concentration-dependently inhibited ADP-, collagen- and high shear stress-induced platelet aggregation, strongly inhibited ATP release from platelets activated by ADP, and enhanced deaggregation of ADP-induced platelet aggregates. In a pro-aggregatory activity study, RGDS or SC-54701A ((*S*)-3-[3-[(4-amidinophenyl)carbamoyl]propionamido]-4-pentynoic acid monohydrochloride) caused prominent small aggregate formation. At a higher concentration, RGDS induced medium and large size aggregates, and SC-54701A induced medium aggregates. In contrast, YM-57029 produced only a few small and no larger size aggregates. Ex vivo ADP-induced platelet aggregation and platelet retention to collagen-coated plastic beads were dose-dependently inhibited by YM-57029 after intravenous bolus injection in guinea pigs. YM-57029 produced dose-dependent antithrombotic effects in carotid artery thrombosis and arterio-venous shunt thrombosis models in guinea pigs at 10 and 30 μg/kg, respectively. At these doses, YM-57029 prolonged template bleeding time. These results suggest that YM-57029 is a potent glycoprotein IIb/IIIa antagonist which has less pro-aggregatory effect. It may be a promising antiplatelet agent for thromboembolic diseases, and a good prototype for developing an orally active compound. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Platelet glycoprotein IIb/IIIa; Antagonist; Pro-aggregatory activity; Antithrombotic agent

## 1. Introduction

The binding of fibrinogen to the glycoprotein IIb/IIIa complex is a final common pathway for platelet aggregation, and inhibition of this step has been found to prevent platelet aggregation induced by all agonists (Plow and Ginsberg, 1989). Therefore, glycoprotein IIb/IIIa antagonists inhibit platelet aggregation and prevent thrombus formation more potently than currently prescribed antiplatelet agents such as aspirin and ticlopidine, because these drugs inhibit only one of several pathways leading to platelet activation (Schror, 1995). Antiplatelet effects have been demonstrated by using anti-glycoprotein IIb/IIIa antibodies (Topol et al., 1994; Kaku et al., 1996), arginine–glycine–aspartic acid (RGD) containing peptides (Scarborough et al., 1991), RGD peptidomimetics (Tcheng et al., 1995; Mousa et al., 1994) and non-peptide compounds (Dooley and Goa, 1999; Topol et al., 1999; Heeschen et al., 1999). The efficacy of the intravenous glycoprotein IIb/IIIa antagonists as adjunctive therapy for percutaneous coronary intervention is well established (Kong et al., 1998). Parenteral glycoprotein IIb/IIIa antagonists, including abciximab (monoclonal antibody), eptifibatide (KGD peptide), and tirofiban (low molecular weight antagonist), are currently in clinical use (Topol et al., 1999).

Orally active glycoprotein IIb/IIIa antagonists may allow more sustained receptor antagonism, thus offering the potential for greater long-term benefit and secondary prevention of recurrent ischemic events. Sequential therapy using an intravenous antagonist followed by an oral antagonist might be desirable. However, recent large-scale, pla-

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PII: S0014-2999(02)01415-2

cebo-controlled, randomized trials of the oral glycoprotein IIb/IIIa antagonists (such as xemilofiban, orbofiban and sibrafiban) have failed to provide commensurate reductions in late composite ischemic endpoints despite potent inhibition of platelet aggregation. Moreover, a worrisome suggestion of increased mortality has been observed (Chew et al., 2001). There is wide inter-individual variation in response to given doses of oral glycoprotein IIb/IIIa antagonists and a relatively narrow therapeutic window in terms of safety and efficacy (Kereiakes et al., 1998; Simpfendorfer et al., 1997). These limitations might explain why oral glycoprotein IIb/IIIa antagonists have not demonstrated sufficient benefits in the conservative medical management of acute coronary syndrome. In addition, it appears that these agents may have a pro-aggregatory effect. This may be caused by dissociation of the drug from the glycoprotein IIb/IIIa receptor, leaving an activated receptor that can then bind fibrinogen and form a platelet aggregate (Cannon, 2000). This effect may be a factor relating to increased mortality. For this reason, a glycoprotein IIb/IIIa antagonist which has less of a pro-aggregatory effect may be of clinical benefit.

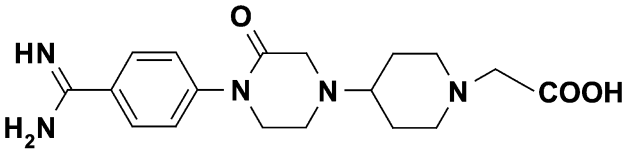
Shear stress-induced platelet aggregation at high shear stress (h-SIPA), which is commonly generated in the stenosed coronary artery, is thought to be a clinically important phenomenon (Goto et al., 1992). A recently invented device for the induction of h-SIPA has enabled the detailed investigation of vWF-glycoprotein Ib and vWF-glycoprotein IIb/IIIa interactions (Ikeda et al., 1991). However, the effects of low molecular weight glycoprotein IIb/IIIa antagonists on h-SIPA have not been reported.

Recently, YM-57029, a potent glycoprotein IIb/IIIa antagonist, has been discovered in our laboratories. In the present study, we first characterized the properties of YM-57029 and compared its effect on fibrinogen binding to purified glycoprotein IIb/IIIa, platelet aggregation, h-SIPA, ATP release, platelet deaggregation, and pro-aggregatory activity with those of SC-54701A (active form of xemilofiban) or RGDS in vitro. Moreover, we examined the antithrombotic effect and the effect on platelet aggregation and bleeding time of YM-57029 in vivo.

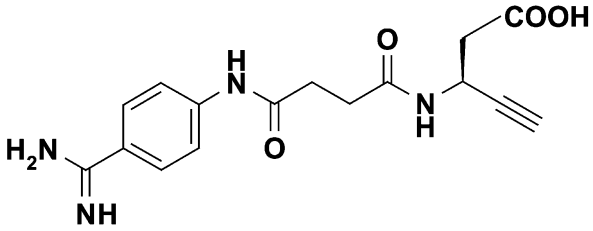
## 2. Materials and methods

### 2.1. Reagents

YM-57029 ({4-[4-(4-carbamimidoylphenyl)-3-oxopiperazin-1-yl]piperidino}acetic acid monohydrochloride trihydrate) and SC-54701A ((*S*)-3-[3-[(4-amidinophenyl) carbamoyl]propionamido]-4-pentynoic acid monohydrochloride) (Nicholson et al., 1995) as a reference compound were synthesized at Yamanouchi Pharmaceutical (Tokyo, Japan). The chemical structures of these compounds are shown in Fig. 1. RGDS peptide was obtained from Peptide Institute (Osaka, Japan). ADP and collagen were purchased from MC Medical (Tokyo, Japan).




YM-57029




SC-54701A

Fig. 1. Structures of YM-57029 and SC-54701A.

### 2.2. Biotinylated fibrinogen binding to purified glycoprotein IIb/IIIa

The glycoprotein IIb/IIIa complex was purified from out-of-date human platelets according to the method of Fitzgerald et al. (1985), except for the use of Sephacryl S-300 (Amersham-Pharmacia, Uppsala, Sweden) gel filtration. Biotinylation of human fibrinogen was performed by mixing 1 mg/ml fibrinogen with 0.2 mg/ml of NHS LC-biotin (Pierce, IL, USA) in 0.1 M $NaHCO_3$ and allowing the reaction to proceed at room temperature for 2 h. After the reaction, the reagent was removed by dialysis against Tris-buffered saline (TBS, pH 7.4). Microtiterplate wells were coated with 1 μg/ml of purified glycoprotein IIb/IIIa in TBS and blocked with bovine serum albumin. Various amounts of the test agent were dispensed into the wells. Ten micrograms of biotinylated fibrinogen in 100 μl were dispensed into the wells, followed by incubation for 3 h at room temperature. After three washes, bound fibrinogen was quantified by the addition of 100 μl of streptavidin-conjugated horseradish peroxidase (1:1000 dilution, Amersham-Pharmacia) followed by incubation for 1 h at room temperature, washing, and color generation with the enzyme substrate, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; BioRad, CA, USA). After stopping the reaction by the addition of 50 μl of 2% oxalic acid, the absorbance at 415 nm was measured using a microplate reader (Model 3550; BioRad).

### 2.3. Species difference in platelet aggregation inhibition

Platelet-rich plasma and platelet-poor plasma were prepared by centrifugation of citrate-anticoagulated blood (3.8% sodium citrate/blood = 1:9) from healthy human volunteers and various animals. Mice, rats, hamsters, guinea pigs, rabbits, beagle dogs, squirrel monkeys, cynomolgus monkeys, and rhesus monkeys were also used in this study.

Platelet counts in the platelet-rich plasma were measured with an automatic blood cell counter (MEK-5158, Nihon Kohden, Tokyo, Japan), and were adjusted to a count of $3 \times 10^5$/μl with platelet-poor plasma. Platelet aggregation was measured using an aggregometer (Hema Tracer 801, MC Medical) by recording the increase in light transmission through a stirred suspension maintained at 37 °C for 5 min. Platelet aggregation in platelet-rich plasma was induced by ADP (human: 2–3 μM; other animals: 20 μM) and collagen (human: 1–2 μg/ml; other animals: 10 μg/ml). The inhibitory effect was calculated by dividing the maximum rate of decrease in absorbance of a mixture containing the test agent by the maximum rate obtained in the presence of buffer alone.

### 2.4. Shear stress-induced platelet aggregation

The inhibitory effect of shear stress-induced platelet aggregation (SIPA) was measured as described previously (Ikeda et al., 1991). A shear stress gradient (6–108 dyn/cm$^2$) was applied to the platelet-rich plasma containing the test drug. Aggregation was continuously monitored by recording the intensity of the light transmitted through the platelet suspension from the beginning of application of the shear force. The inhibitory effect was calculated by dividing the maximum rate of decrease in absorbance of a mixture containing the test agent by the maximum rate obtained in the presence of buffer alone.

### 2.5. ATP release from platelets stimulated by ADP

ADP-induced ATP secretion and platelet aggregation were measured simultaneously by a lumiaggregometer (C560; Chrono-Log, PA, USA). The ATP release reaction was measured by a luciferin–luciferase reaction. YM-57029 or phosphate buffered saline was added to a mixture of luciferin–luciferase reagent (Chrono-Lume®; Chrono-Log) and platelet-rich plasma at 1 min before addition of ADP at a concentration of 20 μM.

### 2.6. Platelet deaggregation

This study was performed using an aggregometer accordant to the method of Freed et al. (1994). The test sample or an equivalent volume of buffer was added 1 min after induction of platelet aggregation in human platelet-rich plasma by ADP (10 μM). Deaggregation was assessed as the decrease in light transmission measured 5 min after addition of the test agent or buffer and expressed as a percentage of the maximal light transmission.

### 2.7. Pro-aggregatory activity

Pro-aggregatory activity was measured as follows. Washed platelets ($5 \times 10^5$/μl) in Tyrode's buffer, pH 7.35, containing 250 ng/ml prostaglandin $E_1$ were incubated with or without various concentrations of test agent at room temperature for 6 min. After the addition of an equal volume of 1% paraformaldehyde, the mixture was incubated at room temperature for 2 h. An equal volume of 0.5 M $NH_4Cl$, 0.15 M NaCl was then added, and the fixed platelets were washed and resuspended in Tyrode's buffer ($2.5 \times 10^5$/μl). Aggregation was induced by the addition of a 1/10 volume of platelet-poor plasma at 37 °C in a laser light scattering aggregometer (AG-10, Kowa, Tokyo, Japan) (Eto et al., 1998). In all cases, the fixed platelet aggregation was completely inhibited by 10 μg/ml of YM337 (anti-human glycoprotein IIb/IIIa mAb), and was not caused by fibrinogen-deficient plasma. This suggests that platelet aggregation is dependent on the interaction between glycoprotein IIb/IIIa and fibrinogen.

### 2.8. Platelet retention to collagen-coated beads in guinea pigs

Male Hartley guinea pigs weighing 309–399 g were anesthetized by an i.p. injection of sodium pentobarbital. One minute after i.v. bolus injection of YM-57029 or saline, blood was withdrawn from the abdominal vein through a column filled with type I collagen-coated plastic beads (Pura Beads Column; ISK, Tokyo, Japan) at a rate of 1.5 ml/min. To measure basal platelet counts, a further sample of blood was withdrawn directly. Each sample was transferred to an EDTA-containing bottle immediately. Platelet counts per microliter in both samples were measured using an automatic blood cell counter, and the percentage of platelets adhering to the beads was calculated.

### 2.9. Ex vivo platelet aggregation study after i.v. bolus injection in guinea pigs

Male Hartley guinea pigs weighing 190–290 g were anesthetized by an i.p. injection of sodium pentobarbital. The test drugs were dissolved in saline and administered by i.v. bolus injection. Five minutes after i.v. bolus injection of the drug, blood was collected from the abdominal vena cava into syringes containing 3.8% citrate, and platelet-rich plasma was prepared by centrifugation to measure platelet aggregation.

### 2.10. Carotid artery thrombosis model in guinea pigs

Experiments were performed modifying the method of Roux et al. (1994). Non-fasted male Hartley guinea pigs weighing 340–510 g were anesthetized by an i.p. injection of sodium pentobarbital. The left carotid artery was carefully dissected free and a 1 mm diameter Doppler probe (DBF10R; Primetech, Tokyo, Japan) was placed to monitor the blood flow. The carotid blood flow was recorded on a polygraph (WI-681G; Nihon Kohden). All agents were given by i.v. bolus injection 1 min before pinching the carotid artery with a surgical forceps. Following thrombus

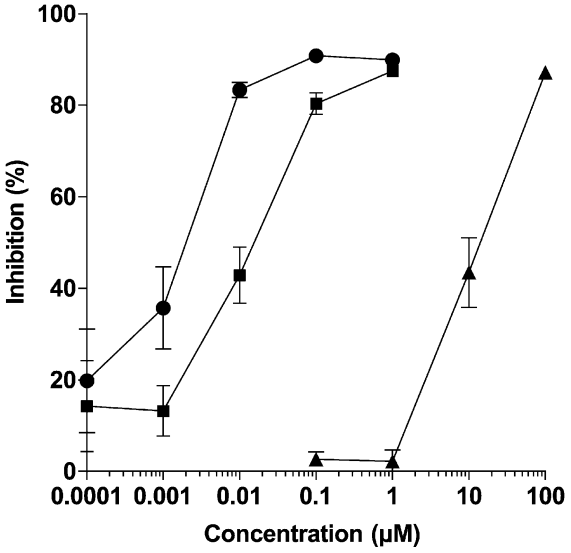



Fig. 2. Effects of YM-57029 (■), SC-54701A (●), and RGDS (▲) on the binding of biotinylated fibrinogen to purified glycoprotein IIb/IIIa. Data represent the mean ± S.E.M. ($n = 5$).

formation, blood flow declined and then stopped. When the flow stopped, the carotid artery was gently shaken until the occlusive thrombus was released and the flow was restored. Every time the flow stopped, this procedure was performed, and the frequency of occlusion over 20 min was measured as the index of thrombus formation.

### 2.11. Arterio-venous shunt thrombosis model in guinea pigs

Experiments were performed using the method of Sato et al. (1998). Non-fasted male Hartley guinea pigs weighing 270–450 g were anesthetized by an i.p. injection of sodium pentobarbital. The left jugular vein and the right carotid artery were cannulated with a 12-cm-long polyethylene tube (o.d. 0.965 mm, PE-50; Clay Adams, NJ, USA). These catheters were connected to the ends of a 10-cm-long polyethylene tube (o.d. 1.52 mm, PE-100; Clay Adams) containing a 2-cm-long copper wire (o.d. 0.3 mm). All agents were given by i.v. bolus injection 1 min before blood was allowed to circulate in the shunt. Ten minutes after blood began circulating in the shunt, the copper wire present in the shunt was gently removed and the attached thrombus was then dissolved in 2 ml of 0.5 N NaOH. The thrombus protein content was measured by photometry using a dye binding assay kit (Bio-Rad) and bovine serum albumin as a protein standard.

### 2.12. Template bleeding time in guinea pigs

Template bleeding time was measured using the method of MacDonald et al. (1994). Non-fasted male Hartley guinea pigs weighing 180–361 g were anesthetized by an i.p. injection of sodium pentobarbital. All agents were administered by i.v. bolus injection 1 min before the measurement of bleeding time. Ear template bleeding time was determined as follows. A template bleeding device (Simplate®; Organon Teknika, Tokyo, Japan) was placed on the dorsal surface of both auricles and triggered. Blood flowing from the incision was gently absorbed with filter paper every 30 s. The time that elapsed until cessation of bleeding was measured and the mean time of the results from both sides was recorded as the bleeding time.

### 2.13. Statistical analysis

In biochemical experiments, $IC_{50}$ or $ED_{50}$ values were calculated from the concentration–response curves in each experiment and data represent the mean of five or six experiments as described elsewhere. In pharmacological studies using animals, the experiments were performed on groups of 3 to 15 animals as described elsewhere and data represent the mean ± standard error of the mean (S.E.M.). Statistical analysis was performed using Dunnett's multiple comparison test for parametric data and Steel's test for non-parametric data. A $P$ value of less than 0.05 was considered significant.

## 3. Results

### 3.1. Effect of YM-57029 on the binding of biotinylated fibrinogen to purified glycoprotein IIb/IIIa

Fig. 2 shows the inhibitory effects of YM-57029, SC-54701A, and RGDS on the binding of biotinylated fibrinogen to purified glycoprotein IIb/IIIa. All these agents concentration-dependently inhibited the binding of biotinylated fibrinogen to purified glycoprotein IIb/IIIa. The $IC_{50}$ values of YM-57029, SC-54701A, and RGDS calculated

Table 1
$IC_{50}$ values of YM-57029 and SC-54701A on ADP- and collagen-induced platelet aggregation in different species

| | $IC_{50}$ values (μM) | | | |
|---|---|---|---|---|
| | YM-57029 | | SC-54701A | |
| Species | ADP | Collagen | ADP | Collagen |
| Human | 0.025 | 0.023 | 0.029 | 0.027 |
| Rhesus monkey | 0.038 | 0.103 | 0.041 | 0.093 |
| Cynomolgus monkey | 0.059 | 0.097 | 0.077 | 0.124 |
| Squirrel monkey | 0.053 | 0.179 | 0.076 | 0.168 |
| Beagle dog | 0.295 | 0.641 | 0.045 | 0.112 |
| Rabbit | 85.4 | 99.7 | 20.3 | 27.5 |
| Guinea pig | 0.179 | 0.325 | 0.048 | 0.115 |
| Hamster | 26.0 | 8.90 | 6.40 | 1.90 |
| Rat | 213 | 81.2 | >1000 | 790 |
| Mouse | 1.90 | 5.00 | 0.56 | 1.50 |

Data represent the mean value of five experiments.

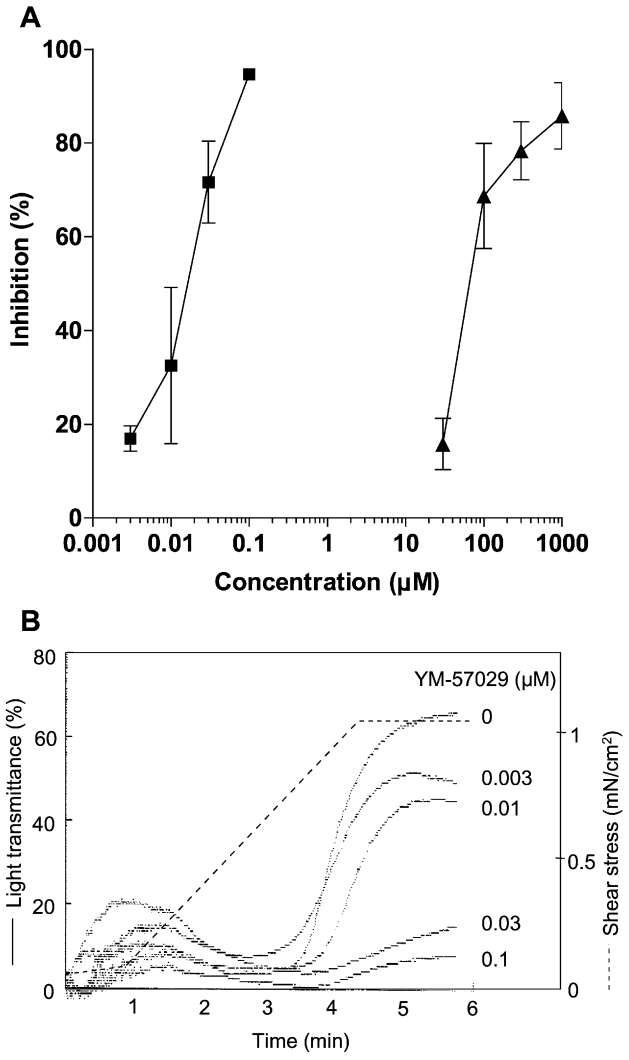



Fig. 3. (A) Concentration–inhibition curves of YM-57029 (■) and RGDS (▲) on h-SIPA. Data represent the mean ± S.E.M. ($n = 5$). (B) Effect of YM-57029 on SIPA in human platelet rich plasma. The results are representative of five separate experiments. The broken line indicates the change in the level of shear stress.

from the concentration–inhibition curves were 14 nM, 1.6 nM, and 10 μM, respectively.

### 3.2. Effect of YM-57029 on platelet aggregation in human and various animals

YM-57029 concentration-dependently inhibited platelet aggregation induced by ADP or collagen in platelet-rich plasmas from human and various animals. Table 1 summarizes the $IC_{50}$ values of YM-57029 and SC-54701A on ADP- or collagen-induced platelet aggregation in each species. In human platelet-rich plasma, YM-57029 inhibited ADP- and collagen-induced platelet aggregation 4000- to 10,000-fold more potently than RGDS ($IC_{50}$ values, ADP: 109 μM; collagen: 236 μM). YM-57029 was most potent with human and monkey platelets, while dog and guinea pig platelets were slightly less sensitive. YM-57029 had relatively little activity against rabbit, hamster, rat, and mouse platelets. In contrast, SC-54701A was potent against guinea pig platelets to the same extent as human and monkey platelets.

### 3.3. Effect of YM-57029 on h-SIPA in human platelet-rich plasma

Fig. 3A shows the inhibitory effects of YM-57029 and RGDS on h-SIPA in human platelet-rich plasma. YM-57029 at 100 nM and RGDS at 1000 μM almost completely inhibited h-SIPA. $IC_{50}$ values of YM-57029 and RGDS were 17 nM and 91 μM, respectively. Fig. 3B shows a representative tracing of shear-induced platelet aggregation in the presence of YM-57029.

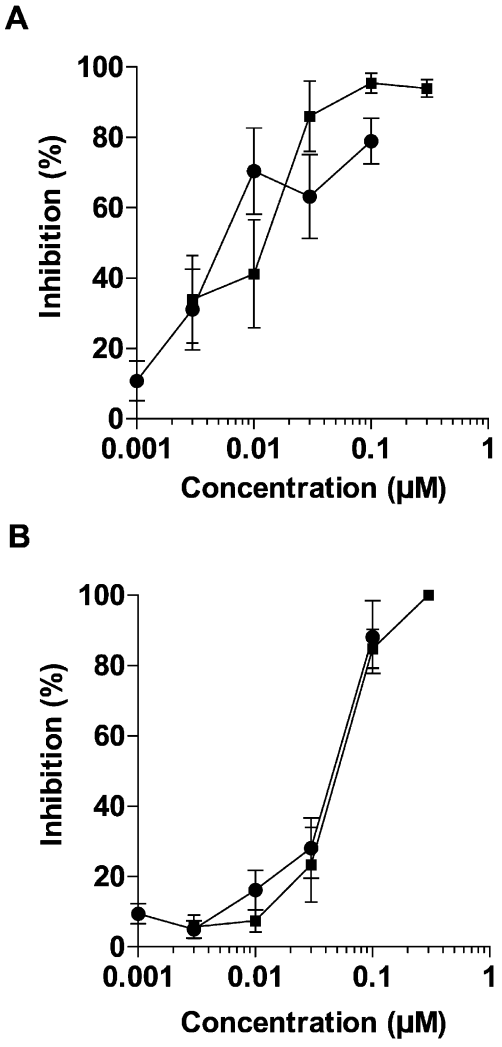



Fig. 4. (A) Concentration–response curves of YM-57029 (■) and SC-54701A (●) on ATP release from platelets stimulated by ADP. Data represent the mean ± S.E.M. ($n = 5$). (B) Inhibitory effect of YM-57029 (■) and SC-54701A (●) on ADP-induced platelet aggregation measured by lumi-aggregometry. Data represent the mean ± S.E.M. ($n = 5$).

### 3.4. Effect of YM-57029 on ATP release from platelets stimulated by ADP

Fig. 4 shows the effect of YM-57029 on ADP-induced ATP secretion (A) and platelet aggregation (B). ATP secretion was concentration-dependently inhibited by YM-57029 and it was almost complete at 30 nM. Platelet aggregation, simultaneously measured in the same experiment, was almost completely inhibited at 100 nM. The $IC_{50}$ values of YM-57029 were 8.4 nM for ATP release and 48 nM for platelet aggregation. SC-54701A exerted almost the same effect as YM-57029.

### 3.5. Effect of YM-57029 on platelet deaggregation of ADP-induced platelet aggregation

Fig. 5 shows the effect of YM-57029 on platelet deaggregation after ADP-induced platelet aggregation. YM-57029 dose-dependently enhanced deaggregation of ADP-induced platelet aggregation, with the same potency as SC-54701A. The $ED_{50}$ values of YM-57029 and SC-54701A were 350 nM and 1 μM, respectively, indicating that these doses were 10- to 20-fold higher than those which inhibited platelet aggregation.

### 3.6. Pro-aggregatory activity

Fig. 6 shows the formation of small (>25 peak count), medium (>400) and large (>1000) size fixed platelet aggregates by platelet-poor plasma. When intact platelets were incubated with 0.1 mM RGDS (nearly equivalent to the $IC_{50}$ value of RGDS on ADP-induced platelet aggregation) or 0.1 μM SC-54701A (about 4-fold higher concentration than its $IC_{50}$ value on ADP-induced platelet aggregation) prior to fixation and wash-out of the agent, small aggregate formation was prominent. After treatment with 1 mM RGDS, medium and large size aggregates appeared. One micromolar SC-54701A also caused medium aggregates. In the case of YM-57029, concentrations of 0.1 μM (a 4-fold higher concentration than its $IC_{50}$ value on

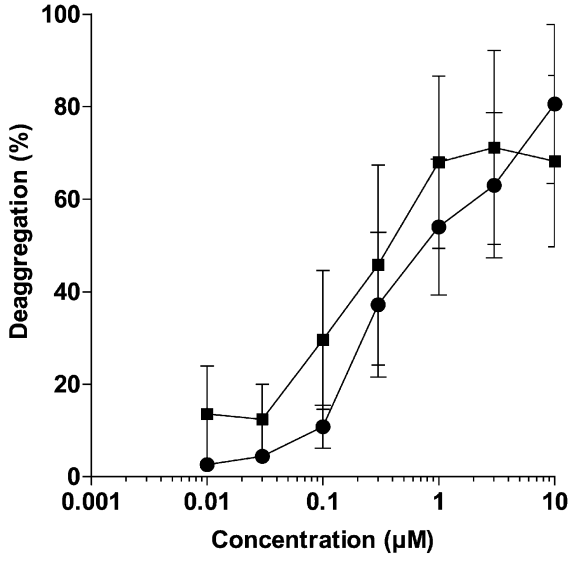



Fig. 5. Effects of YM-57029 (■) and SC-54701A (●) on deaggregation of ADP-induced platelet aggregation. Data represent the mean ± S.E.M. ($n=5$).

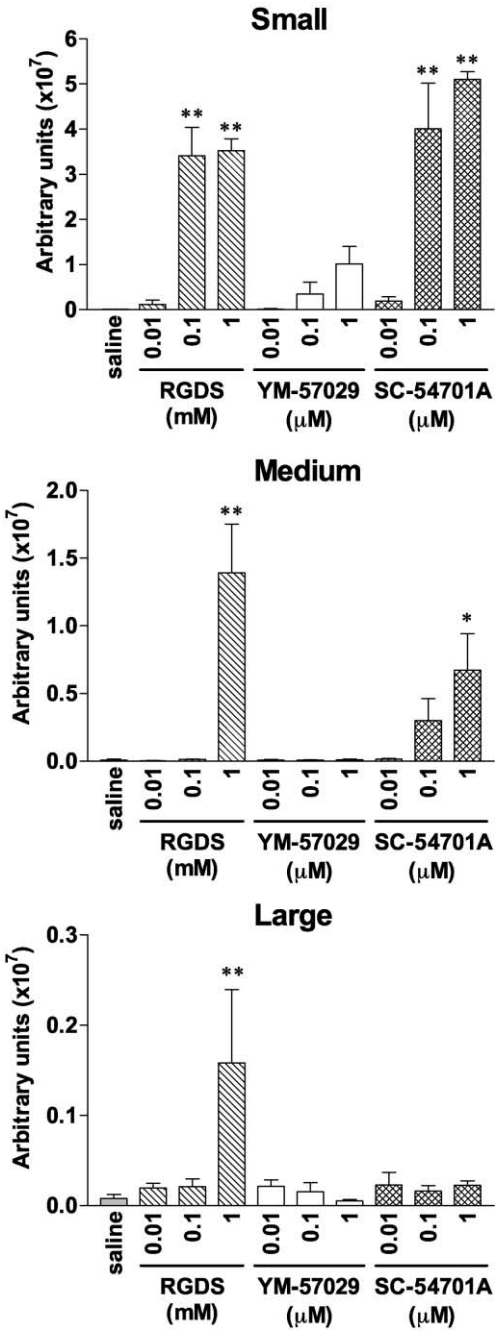



Fig. 6. Pro-aggregatory effects of RGDS, YM-57029, and SC-54701A. Data represent the mean ± S.E.M. ($n=4$). * $P<0.05$, ** $P<0.01$ vs. saline control by Dunnett's multiple comparison test.

ADP-induced platelet aggregation) and 1 μM produced only a few small aggregates with no significant difference and no larger size aggregates.

### 3.7. Effect of YM-57029 on platelet retention to collagen-coated beads in guinea pigs

YM-57029 dose-dependently inhibited platelet retention to collagen-coated beads in guinea pigs 1 min after i.v. bolus injection. YM-57029 significantly inhibited platelet retention at 10 and 30 μg/kg by 40% and 84%, respectively (data not shown).

### 3.8. Ex vivo platelet aggregation, antithrombotic effect in vivo, and bleeding time

Fig. 7 shows ex vivo platelet aggregation induced by ADP, the antithrombotic effects in the carotid artery thrombosis and arterio-venous shunt models, and the effect on template bleeding time after i.v. bolus injection of YM-57029. YM-57029 dose-dependently inhibited platelet aggregation in guinea pigs. YM-57029 almost completely inhibited platelet aggregation 5 min after an i.v. bolus injection of 30 μg/kg.

YM-57029 dose-dependently inhibited thrombus formation in both the carotid artery thrombosis model and the arterio-venous shunt model. YM-57029 produced significant antithrombotic effects at 10 μg/kg and more in the carotid artery thrombosis model and at 30 μg/kg and more in the arterio-venous shunt model.

YM-57029 dose-dependently prolonged bleeding time. YM-57029 significantly prolonged bleeding time at 10 μg/kg.

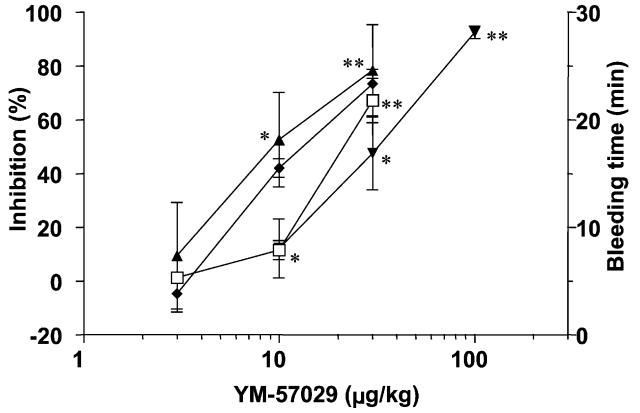



Fig. 7. Effects of YM-57029 on ADP-induced platelet aggregation (♦), a carotid artery thrombosis model (▲), an arterio-venous shunt thrombosis model (▼), and template bleeding time (□) after i.v. bolus injection in guinea pigs. YM-57029 was injected at 0.003, 0.01, 0.03, and 0.1 mg/kg. The experiments were performed on groups of three animals for ex vivo platelet aggregation, six animals for the thrombosis model, 7–12 animals for the bleeding model, and data represent the mean ± S.E.M. * $P<0.05$, ** $P<0.01$ vs. saline control by Dunnett's multiple comparison test.

## 4. Discussion

In this study, we found that YM-57029 is a potent glycoprotein IIb/IIIa antagonist which strongly inhibits various platelet functions including agonist-induced platelet aggregations, h-SIPA and ATP-release reactions, and which enhances platelet deaggregation after ADP-induced platelet aggregation.

YM-57029 inhibited the binding of biotinylated fibrinogen to purified glycoprotein IIb/IIIa 700-fold more potently than RGDS. The inhibitory potency of YM-57029, however, is 8- to 9-fold less potent than that of SC-54701A. The spatial configuration of RGD depends on the tertiary and secondary structures of the molecule (Huang et al., 1987). Our results imply that the spatial configuration of YM-57029 or SC-54701A contributes to the much higher potency than that of a linear RGDS peptide.

YM-57029 strongly inhibited ADP- and collagen-induced platelet aggregation in platelet-rich plasmas samples from human and monkeys. The inhibitory potency of YM-57029 on platelet aggregation in human platelet-rich plasma was almost the same as that of SC-54701A. These results did not reflect those from the binding studies. This is probably because of differences in experimental conditions between platelet aggregation studies and binding studies, such as shear stress and the existence of plasma protein. Interestingly, SC-54701A, not YM-57029, had almost the same inhibitory potency against beagle dogs and guinea pigs platelet-rich plasma as against human platelet-rich plasma samples. The interspecies differences of non-peptide glycoprotein IIb/IIIa antagonists on platelet aggregation have been reported (Bernat et al., 1999; Cox et al., 1992). Studies suggest that the species difference is probably due to differences in binding of the compounds to the platelet receptors, and not in the structure of fibrinogen (Hoffmann et al., 1997; Bernat et al., 1999).

vWF-mediated platelet aggregation occurs when shear stress is applied to platelet-rich plasma or washed platelets in the absence of any exogenous agonist (Peterson et al., 1987). Previous studies using a SIPA device indicate that platelet aggregation under high shear stress is totally dependent on the presence of plasma vWF and glycoprotein IIb/IIIa, but is independent of the presence of plasma fibrinogen. The molecular mechanism of h-SIPA is now partially understood: the initial binding of vWF to glycoprotein Ib leads to $Ca^{2+}$ influx into the platelets, followed by additional vWF binding to activated glycoprotein IIb/IIIa. SIPA at high shear stress appears to be clinically important because these circumstances are very likely generated in the stenosed coronary artery in vivo (Goto et al., 1992; Folts et al., 1982). In the present study, YM-57029 inhibited h-SIPA at the same potency as agonist-induced platelet aggregations in human platelet-rich plasma. These findings suggest that YM-57029 can effectively inhibit the interaction of vWF with glycoprotein IIb/IIIa under high shear stress.

Platelet granular secretion is an important physiological event that may occur with platelet activation and aggregation (Levine et al., 1981), but limited information is available about the effects of glycoprotein IIb/IIIa antagonists on platelet granular secretion. Our results showed that YM-57029 dose-dependently inhibited ADP-induced ATP secretion, and its inhibitory potency on ATP release was more potent than on platelet aggregation. Similar results have been reported by Freed et al. (1994). However, controversial results that there was no direct correlation between platelet aggregation inhibition and platelet secretion inhibition by glycoprotein IIb/IIIa antagonists have been reported (Dickfeld et al., 2001; Klinkhardt et al., 2000; Tsao et al., 1997). Since these kinds of studies depend on the experimental conditions, such as platelet agonists used, concentration of the agonist, and racial difference of volunteers, caution should be taken in interpreting these results.

YM-57029 dose-dependently induced platelet deaggregation. The deaggregatory effect of YM-57029 supports its competitive inhibition because platelet aggregation is initiated by fibrinogen binding to the platelet glycoprotein IIb/IIIa receptor in a reversible manner. Our results are consistent with those of Satoh et al. (1993) and Haskel and Abendschein (1989). Although we examined only the effect of YM-57029 on deaggregation in vitro, further experiments are needed to assess in vivo the potential significance of the deaggregatory effects of this agent.

Because the pro-aggregatory activity of glycoprotein IIb/IIIa antagonists is thought to be a factor relating to the mortality increase in clinical trials (Cannon, 2000), the pro-aggregatory effects of YM-57029 were compared with SC-54701A using a laser light scattering aggregometer. This system makes it possible to measure the formation of small, medium, and large size platelet aggregates separately (Tohgi et al., 1996). Even at high concentrations YM-57029 produced only a few small aggregates and no larger size aggregates. In contrast, after incubation of intact platelets with 0.1 mM RGDS or 0.1 μM SC-54701A, small aggregate formation was prominent. In addition, after treatment with 1 mM RGDS, medium and large size aggregates appeared, and 1 μM SC-54701A also caused the formation of medium aggregates. These data suggest that YM-57029 has relatively weak pro-aggregatory activity compared with RGDS or SC-54701A. There are two possibilities to explain the mechanism by which YM-57029 has little pro-aggregatory activity. One is that YM-57029 has a slow off-rate and still exists on the platelet even after washing. But this is unlikely because the number of small aggregates tended to increase at higher concentrations of YM-57029, although this was not statistically significant. Another possibility is that YM-57029 binds to glycoprotein IIb/IIIa receptors with little effect on the conformational change of glycoprotein IIb/IIIa to the active form. Further study will be needed to fully delineate the mechanism of the lesser pro-aggregatory activity of YM-57029 and to determine whether this property is correlated with clinical outcome.

In this study, we showed that YM-57029 dose-dependently inhibits ex vivo platelet retention to collagen beads as well as in vivo thrombus formation in the carotid artery platelet-rich thrombosis and arterio-venous shunt thrombosis models in guinea pigs, and these effects are consistent with the inhibition of ex vivo platelet aggregation and the prolongation of template bleeding time. Bleeding time and platelet retention to collagen beads are considered reliable indicators of platelet function and are used clinically to identify patients with bleeding tendencies (Hellem, 1960; Harker and Slichter, 1972). Furthermore, YM-57029 significantly inhibited in vivo thrombus formation in the carotid artery thrombosis model in guinea pigs with 3-fold more potency than it did in the arterio-venous shunt thrombosis model. This study showed that the antithrombotic effects of YM-57029 were more pronounced in the carotid artery thrombosis model, in which the thrombus formed is made almost entirely of platelets (Roux et al., 1994), than in the arterio-venous shunt model, in which the thrombus is made of both platelets and fibrin (Peters et al., 1991). Furthermore, YM-57029 had no significant antithrombotic effects in stasis-induced venous thrombosis in guinea pigs even at a dose of 300 μg/kg (data not shown), in which the thrombus formed is made almost entirely of fibrin with few platelets (Reyers et al., 1980). These results are expected, considering the mechanism of action of glycoprotein IIb/IIIa antagonists, and are consistent with other studies (Coller et al., 1989; Shebuski et al., 1990; Cook et al., 1993; Dooley and Goa, 1999).

In conclusion, YM-57029 is proved to be a potent glycoprotein IIb/IIIa antagonist both in vitro and in vivo, and it has little pro-aggregatory effect. These results suggest that YM-57029 may be a promising parenteral antiplatelet agent and a good prototype in the development of an orally active compound without prothrombotic activity.

## Acknowledgements

We thank Dr. Noboru Satoh, Dr. Takashi Fujikura, and Dr. Toichi Takenaka for their interest and encouragement, and Dr. A. Parker Doke for editing the manuscript.

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