Drugs of the Future 1998, 23(3): 291-299

CCC: 0377-8282/98

Review Article

Rational drug design of the fibrinogen inhibitors FK633 and FR158999

Akito Tanaka[1*], Hiroyoshi Sakai[2], Takatoshi Ishikawa[2], Toshiaki Aoki[3], Yukio Motoyama[4], Hideaki Fujiwara[5], Hisashi Takasugi[2].

[1]Basic Research Laboratories, [2]Medicinal Chemistry Research Laboratories and [3]Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan; [4]Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., Ibaraki, Japan; [5]School of Allied Health Sciences, Osaka University Faculty of Medicine, Suita, Japan. [*]Correspondence

CONTENTS

Introduction

Uncontrolled platelet aggregation and platelet adhesion to the subendothelium of damaged blood vessels causes life-threatening diseases such as myocardial infarction (1), transient ischemic attack (1) and unstable angina (2). Pharmacological and medicinal chemistry studies have elucidated both physiological mediators and synthetic inhibitors of the platelet aggregation cascade. One novel approach to thrombosis treatment and prevention arose from the remarkable finding that a small amino acid sequence, Arg-Gly-Asp-Ser (RGDS), mediates the von Willbrand factor-platelet interaction (3). Subsequent studies found that numerous adhesion reactions are RGD-dependent as well (4). During platelet aggregation, the RGD sequence of fibrinogen binds activated heterodimer glycoprotein receptor GPIIb/IIIa on the surface of platelets (5). This is thought to be the most critical step in platelet aggregation, and as a result, a number of different types of fibrinogen inhibitors have been reported in the past several years. Among them, GPIIb/IIIa antibody (c7E3) effectively enhances the rate of thrombolysis with tissue plasminogen activator (t-PA) and prevents reocclusion (6). It also reduces clinical restenosis after coronary angioplasty whereas nonfibrinogen inhibitors have failed (7, 8). The general use of this antibody, however, appears to be limited by its immunogenecity (9) and slow reversibility (6). Hence, various chemical approaches have been studied in order to develop small molecule fibrinogen inhibitors (10-13).

The purpose of this article is to describe the design, synthesis and evaluation of two kinds of fibrinogen inhibitors. The first, which includes our clinical candidate, 4-(4-amidinophenoxy)butanoylaspartylvaline (FK633, **3g**), an agent for intravenous administration, was originally designed from a putative active structure of the RGD peptide generated using computer simulations (14). The second, including 4-(4-amidinophenoxy)butanoylaspartylvalylthiomorpholine 1,1-dioxide, the successor compound (FR158999, **6f**) to FK633, is orally active and was rationally designed based on results of metabolic studies of FK633 and related derivatives (15).

A QSAR study on FK633 and its derivatives is also described. Our QSAR studies using several different parameters, including a novel descriptor, the logarithm of the partition coefficient micelle/water (logPmw) (16), indicated that an important factor for inhibitory activity is not only molecular shape and hydrophobicity but also membrane affinity (17).

Design and synthesis of FK633 derivatives

At the beginning of this study, we designed an ideal lead compound (**2**) (10) from the structures of the RGD peptide and reported fibrinogen inhibitors (10-13). Compound **2** consists of three moieties: 1) two functional groups vital for activity (benzamidine and Asp), 2) a spacer moiety which correctly positions the end groups, and 3) an amino acid derivative (AA) at its carboxy terminal (C-terminal).

We carried out computer simulations to select the length of the spacer moiety of compound **2** (m in Fig. 1)

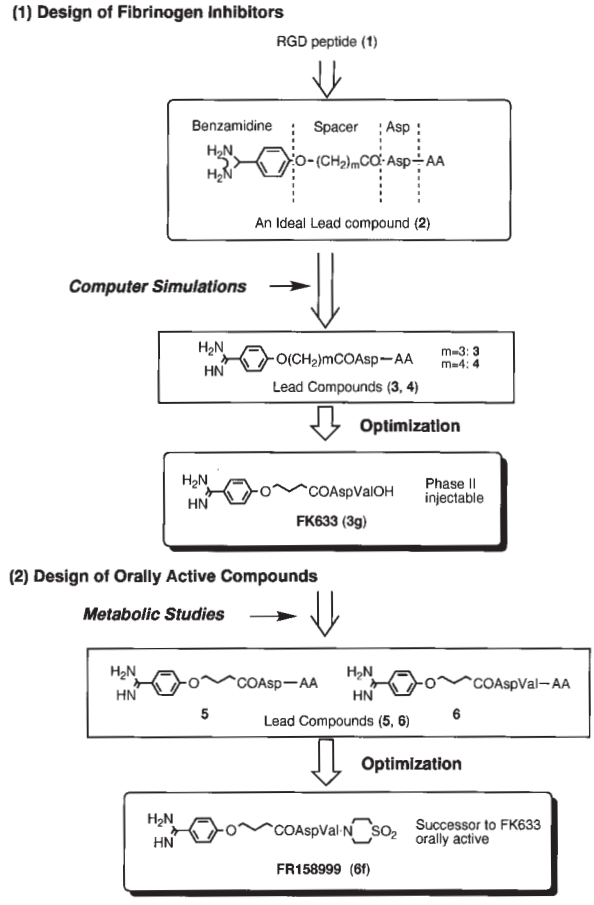


Fig. 1. Design of fibrinogen inhibitors (FK633, FR158999).

before starting the synthesis of derivatives. In computer simulations (18), each derivative of **2** (m=2, 3, 4 or 5) was superimposed on the RGD peptide to overlap the position of the two key functional groups (amidino and carboxylic acid) of both compounds (broken lines, Fig. 2a). It is very important when performing computer simulation to select the active conformation of the reference compound, the RGD peptide in this case, from the large number of possible conformations existing at the energy level of body temperature. In our study, we postulated a type II' β-turn (19a) with the Gly positions at (i+1)th (19b) as the active conformation of the RGD peptide due to the following reasons: 1) NMR study of the RGD protein, decorsin, showed that the solution structure of the RGD moiety is a distorted type II' β-turn (20); 2) assuming a type II' β-turn as the active conformation of the RGD sequence, the evolutionary significance of conservation of the Gly residue in the RGD sequence becomes clear. Thus, Gly is conserved in a wide range of adhesion proteins, independent of species (4), in spite of a lack of functional groups, whereas the other two conserved amino acids (Arg and Asp) have functional groups that are able to interact with the receptor (GPIIb/IIIa) through strong hydrogen bonds. From this it can be supposed that Gly plays a critical role in maintaining the active conformation. While many possible conformations are possible, the type II' β-turn is thought to be the most suitable as the active conformation since it is well-known that Gly often appears in the (i+1)th position of type II' β-turns (21). Hence, we decided to set the active conformation of the RGD sequence to be a type II' β-turn in which Gly is positioned at (i+1)th in our computer simulations.

Our computer simulation studies provided two energetically indistinguishable moieties (Fig. 2b): m=3 and m=4, in which the two vital binding groups occupy similar positions with reasonable conformations (energy loss of these conformations was less than 5 kcal/mol compared with the global minimum conformations). As a result, we decided to synthesize 4-(4-amidinophenoxy)butanoylaspartic acid (**3**, m=3) and 3-(4-amidinophenoxy)pentanoylaspartic acid derivatives (**4**, m=4) as lead compounds in this study, and modified only the C-terminal moiety of these compounds (AA moiety of compounds **3** and **4**) in the optimization process, since modification of the C-terminal moiety is known to be effective for increasing inhibitory activity (22).

The inhibitory activities of compounds prepared in this study against human platelet aggregation induced by ADP were measured initially (23). Potent compounds from the first screening were then tested in an inhibition study of fibrinogen attachment to platelets to confirm that the antiplatelet activity of these compounds was due to inhibition of fibrinogen binding.

The inhibitory activities of compounds examined in this study are shown in Table I. The potency of compounds with m=3 (**3a-g**) was similar to that of compounds with m=4 (**4a-e**), which are consistent with the results of our computer simulations, *i.e.*, compounds with m=3 or m=4 are essentially indistinguishable (24). The inhibitory activity in the fibrinogen binding assay for these compounds was similar to the results from the primary screening, which indicated that the antiplatelet activity of these compounds is due to inhibition of fibrinogen binding to GPIIb/IIIa on the surface of platelets. Among these compounds, 4-(4-amidinophenoxy)butanoylaspartylvaline (FK633) exhibited potent inhibitory activity (IC_{50} = 0.10 μM) and was selected for further evaluation because of its potent antiplatelet activity based on fibrinogen binding inhibition, low cost of synthesis, low toxicity, absence of side effects *in vivo* and high solubility.

In further studies, FK633 (0.1 mg/kg i.v.) significantly suppressed *ex vivo* ADP-induced platelet aggregation (> 40% inhibition, Table II) but did not prolong template bleeding time at this dosage. However, it did prolong bleeding time at a dose of 0.32 mg/kg. FK633 also prevented thrombus formation at stented and injured coronary artery after bolus injection of 0.1 mg/kg in dogs (Fig. 3) (25). These results indicate that FK633, currently undergoing clinical phase II trials, should be effective as an antiplatelet drug.

QSAR studies on FK633 derivatives

Many studies on structure-activity relationships of the RGD moiety by NMR and x-ray crystallography methods

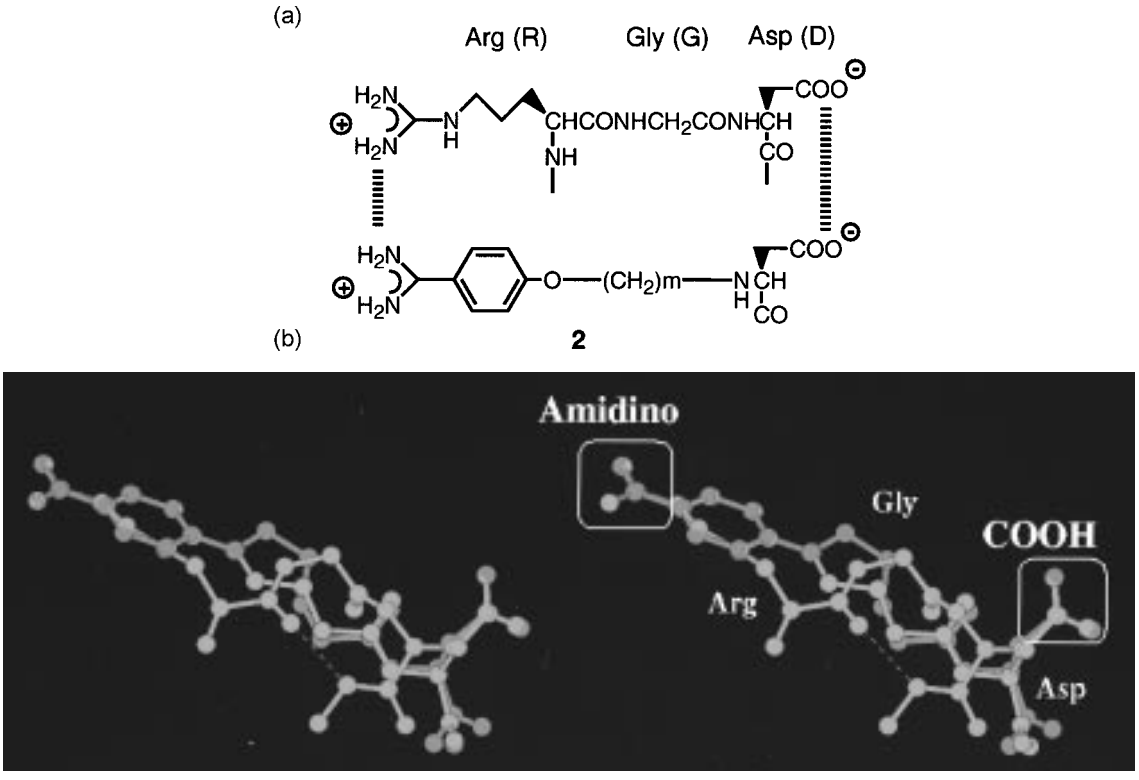


Fig. 2. (a) Structural relationship between RGD peptide and **2**. (b) Stereo view of two energetically indistinguishable conformations of compound **2** (m=3, yellow; m=4, cyan) which were obtained by means of superimposition studies with type II' β-turn of the RGD sequence (pink). The Gly positions at the (i+1)th of type II' β-turn (see text). AA moiety of compound **2** is not shown.

have been performed to understand the active conformation of the RGD moiety for rational drug design of novel fibrinogen inhibitors, and much information has been accumulated in the past several years (26-30). However, few QSAR studies on moieties other than the RGD have been carried out, despite the fact that these moieties, especially the C-terminal moiety (AA in this study), significantly affect inhibitory activity (22). Thus, we decided to carry out QSAR studies on FK633 and its derivatives, modified only at the C-terminal position (Table I), to understand the role of the C-terminal moiety.

QSAR studies using three descriptors, logPmw, CLOGP (31, 32) and STERIMOL (32), were performed. The logPmw, the logarithm of the partition coefficient

*Table I: Structures and inhibitory activities of FK633 derivatives (**3**, **4**) with their QSAR parameters.*

			Inhibitory activities IC_{50} (μM)		QSAR parameters	
Compd.	m[a]	AA[a]	Platelet aggregation	Fibrinogen binding	logPmw	CLOGP
3a	3	NleOH	0.076 ± 0.07[b]	NT[c]	1.58	0.79
3b	3	IleOH	0.14 ± 0.02	NT	1.47	0.66
3c	3	LeuOH	0.13 ± 0.02	NT	1.47	0.66
3d	3	g-Me-LeuOH	0.23 ± 0.04	NT	1.59	1.06
3e	3	Tyr(Me)OH	0.12 ± 0.06	NT	1.96	0.54
3f	3	TyrOH	0.16 ± 0.01	NT	1.74	-0.05
3g (FK633)	3	ValOH	0.10 ± 0.01	0.088 ± 0.01	1.29	0.13
4a	4	SerOH	12 ± 0.09	NT	0.90	-1.34
4b	4	ValOH	0.23 ± 0.05	0.23 ± 0.05	1.32	0.66
4c	4	TyrOH	0.64 ± 0.13	0.64 ± 0.01	1.82	0.48
4d	4	Tyr(Me)OH	0.27 ± 0.08	0.27 ± 0.08	2.00	1.07
4e	4	TyrOMe	4.5 ± 0.9	NT	2.09	0.62
RGDS			75 ± 12	95 ± 10		

[a]See Fig. 1; [b]mean ± SD; [c]NT= not tested.

Table II: Inhibitory effects of FK633 on ex vivo *ADP-induced platelet aggregation and template bleeding time after intravenous administration (0.1 mg/kg i.v.)*

Platelet activity (% inhibition)				Bleeding time (min)			
0	0.5	1	2h	0	0.5	1	2h
0 ± 0[a]	47 ± 13	39 ± 9	6 ± 18	5.2 ± 0.4	6.0 ± 0.3	6.5 ± 0.6	4.7 ± 0.2

[a]Mean ± SD.

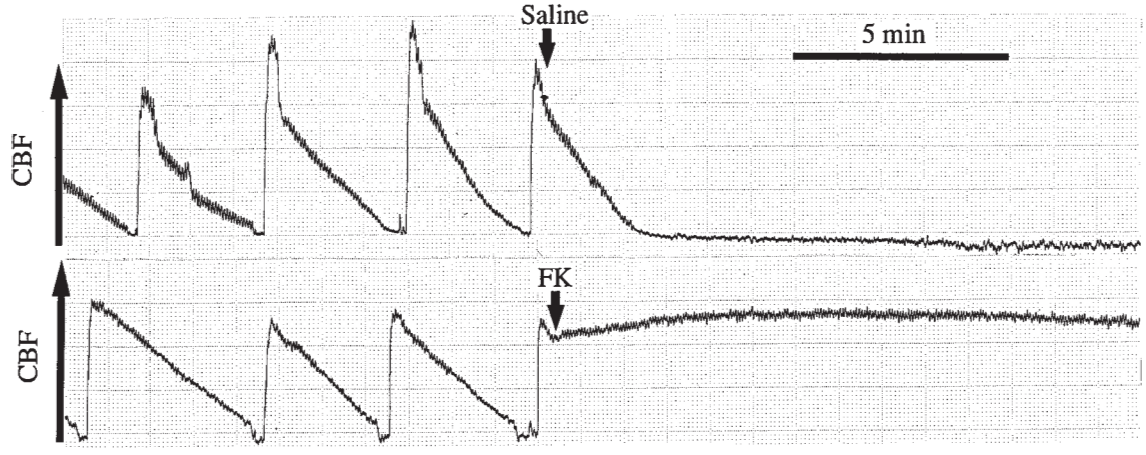


Fig. 3. Effect of FK633 on tracings of coronary blood flow (CBF) after stenosis of the injured circumflex artery. Cycles of blood flow reduction, each of which was terminated by mechanical shaking loose from the thrombus, were observed during the pretreatment periods. FK633 at 0.1 mg/kg i.v. effectively prevented the thrombus formation and maintained CBF, in contrast to the coronary artery which was completely occluded in saline-treated dogs.

micelle/water, was recently introduced into QSAR studies (16). It is considered useful to describe a compound's affinity to a biomembrane and as a hydrophobic descriptor in QSAR studies, since the micelle/water system, used to estimate logPmw values, is recognized as a suitable model of the biomembrane/water interface (Fig. 4) (16, 17). This is in contrast to the use of a miscible organic solvent such as *n*-butanol for estimation of the logP values. CLOGP is widely used as a parameter for a compound's hydrophobicity because of its accuracy and ease of calculation (31). The reasons for adopting these hydrophobic parameters were that the physical properties of the compounds seemed to be especially important for inhibitory activity, and few studies have been reported on the relationship between the physical properties and antiplatelet activity of fibrinogen inhibitors, although both structural and physical properties of bioactive compounds are generally important for potent inhibitory activity. LogPmw and CLOGP values of compounds examined in this study are shown in Table I. STERIMOL parameters are often used as descriptors for molecular shape of compounds to understand the structural properties required for potent activity.

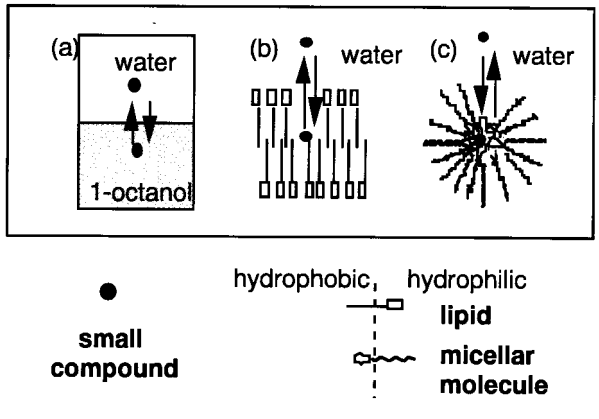


Fig. 4. Partition of small compounds in (a) 1-octanol/water (logP), (b) membrane/water (*in vivo*) and (c) micelle/water (logPmw) systems.

A plot of observed logPmw values *versus* antiplatelet activity (pIC_{50}) for FK633 derivatives is shown in Figure 5a and indicates that this relationship looks like a quadratic curve. A statistical calculation on Figure 5a was carried out and resulted in:

$$pIC_{50} = -4.36(\pm1.88)(logPmw)^2 + 13.7(\pm5.85)(logPmw) - 3.84(\pm4.42) \quad (A)$$

$$= -4.36(logPmw-1.57)^2 + 6.93 \quad (B)$$

n=12, s=0.368, F=14.1**, r=0.871

The good correlation coefficient (s=0.368, F=14.1**, r=0.871) in equation (A) shows that the antiplatelet activities of the fibrinogen inhibitors (pIC_{50}) depend on their logPmw values. The values in parentheses in equation

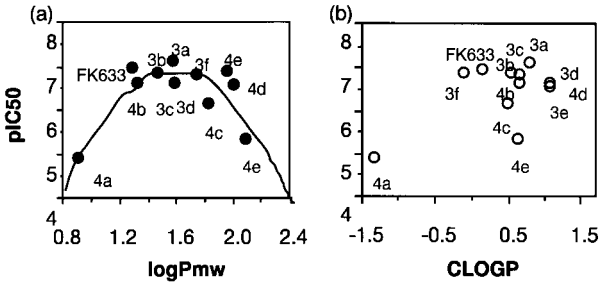


Fig. 5. Plots of fibrinogen inhibitory activities (pIC_{50}) of FK633 derivatives with (a) logPmw and (b) CLOGP.

(A) indicate 95% confidence intervals. Equation (B) indicates that a compound having a 1.57 logPmw value exerts the maximum antiplatelet activity, which agrees with the experimental results; that is, the most potent compound **3a**, whose logPmw value is 1.58, is the one nearest to the 1.57 logPmw value. Therefore, all compounds having suitable logPmw values (1.3-1.7), such as FK633 and compounds **3a-d**, **3g** and **4b**, showed potent antiplatelet activity while compounds **4a** and **4e**, whose logPmw is far from the 1.57 logPmw value, are not potent.

We next carried out QSAR studies using STERIMOL parameters and CLOGP values. QSAR studies on the AA moiety (33) using STERIMOL parameters were unsuccessful (data not shown), indicating that the shape of the AA moiety is not important for antiplatelet activity and is not severely recognized at the binding pocket of the receptor. A QSAR study using CLOGP values also resulted in a poor relationship (Fig. 3b). The difference between QSAR results using logPmw and those using CLOGP was very interesting to us since both are indicators of the same hydrophobic property of compounds. Based on these results, we concluded that the important feature of the AA moiety of FK633 derivatives for their inhibitory activity is not their molecular shape (STERIMOL) or simple hydrophobicity (CLOGP), but their membrane affinity (logPmw).

We postulated that there were two possibilities for the role of the C-terminal moiety: 1) the AA moiety plays an important role in interacting with the binding pocket of the GPIIb/IIIa receptor through hydrophobic interactions, and 2) potent inhibitors bind to the receptor via the biomembrane (route A in Fig. 6), and the AA moiety thus functions as an anchor by which the inhibitors interact with the biomembrane. We eliminated the first possibility because of two reasons. First, it is not considered necessary for inhibitors to interact with the membrane if these compounds immediately bind directly to the receptors (34); however, QSAR studies using only logPmw displayed good results. And second, QSAR studies using CLOGP should give similar results as studies using logPmw and some structural requirements (STERIMOL parameters) should be observed in the first possibility.

According to this hypothesis, the reason for inactivity of compounds whose logPmw values are far from the maximum value (1.57 logPmw) can be interpreted as follows. Compounds with a low logPmw value, such as compound **4a**, must bind to the receptor directly from the solution state due to low membrane affinity. In such a case, it is necessary to adapt the active conformation in the solution state for effective binding with the GPIIb/IIIa receptor, a statistically unlikely event (35). On the other hand, it is difficult for a compound whose logPmw is very high, such as compound **4e**, to release itself from the membrane to bind to the binding pocket due to very high membrane affinity, and thus results in overall low inhibitory activity. This hypothesis has not yet been experimentally confirmed, although we are currently examining it in detail.

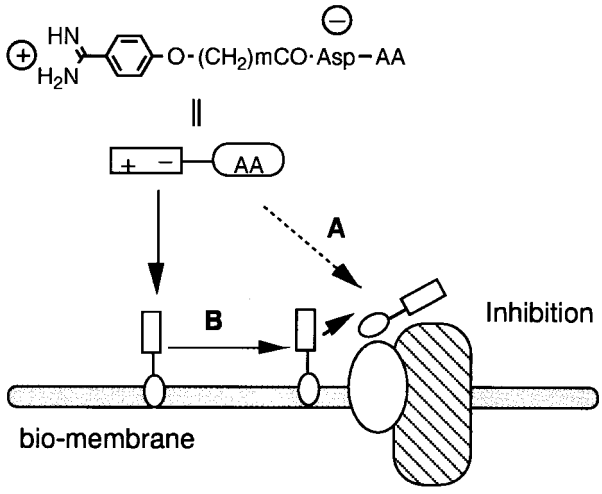


Fig. 6. Two possible inhibitory pathways from solution state to the binding state and tentative route of compounds (route B). In route A, inhibitors immediately bind to the GPIIb/IIIa receptor, and inhibitors bind to the receptor via biomembrane in route B (see text for details).

Metabolic studies on FK633 derivatives

FK633 displayed potent *in vivo* antiplatelet activity, inhibiting platelet-dependent coronary artery thrombosis in dogs following intravenous infusion (Fig. 3), indicating that it is potentially clinically useful for acute treatment. On the other hand, orally active antiplatelet drugs are clinically necessary for the treatment of chronic diseases. Thus, we assessed the bioavailability of FK633 in a follow-up study, in which its antiplatelet activities were measured after oral administration in guinea pigs, rats and dogs (Table III).

Interestingly, FK633 showed potent antiplatelet activity in guinea pigs even at a dose of 0.32 mg/kg p.o., even though it is generally considered that peptidic compounds such as FK633 are not effective after oral administration. The bioavailability of FK633 in rats and dogs, however, is insufficient (Table III). Thus, our next task focused upon obtaining compounds which were effective after oral administration, not only in guinea pigs but also in rats and dogs.

The antiplatelet activity of FK633 in rats after oral administration was increased 3-fold (36) by co-

Table III: Inhibitory activities ex vivo *of FK633 in guinea pigs, rats and dogs after oral administration.*

Dogs				Rats				Guinea pigs			
Dose	Inhibition (%)			Dose	Inhibition (%)			Dose	Inhibition (%)		
(mg/kg)	1	2	3h	(mg/kg)	0.5	1	2h	(mg/kg)	1	3	6h
3.2	19 ± 17[a]	30 ± 13	10 ± 6	10	24 ± 6	18 ± 6	4 ± 70	32	70 ± 19	NT[b]	NT
32	58 ± 7	29 ± 5	18 ± 13	20	45 ± 8	33 ± 8	20 ± 17	1.0	99 ± 15	34 ± 20	NT
								3.2	100 ± 8	64 ± 18	19 ± 18

[a]Mean ± SD; [b]NT= not tested.

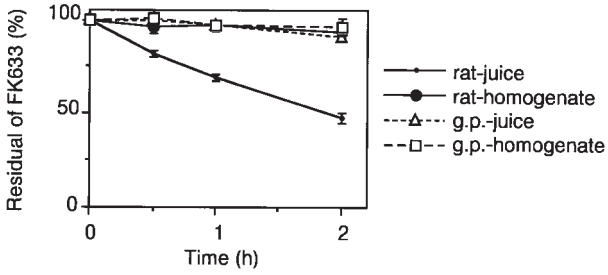


Fig. 7. Stability of FK633 at 37 °C in intestinal juice and homogenate of intestine of rats and guinea pigs (g.p.)

administration with the known peptidase inhibitor, bacitracin (37). This indicated that the low bioavailability of FK633 in rats and in dogs arose from hydrolysis by peptidase in intestinal juice and/or cells, whereas it is stable in that of guinea pigs, resulting in high bioavailability. In order to assess this hypothesis, we tested the stability of FK633 in intestinal juice and homogenate of intestinal cells of rats and guinea pigs (Fig. 7). This study showed that FK633 was degraded only in the intestinal juice of rats and was completely stable in that of guinea pigs and in homogenates of intestinal cells of rats and guinea pigs, which supports our supposition (38) and indicates that design of novel FK633 derivatives which are stable in the intestinal juice of rats could afford compounds with the desired profile.

Upon inspection of the structure of FK633, there are three moieties (A, B and C) which could be considered responsible for hydrolysis in intestinal juice (Fig. 8). In order to investigate the structure stability relationships of these moieties to aid rational design of orally active compounds, we synthesized compounds whose carboxyl group (C), which could be recognized by peptidases such as carboxypeptidases, was removed (**5a-b**) or replaced with carboxamide (**6a**), or whose amide bond (A), which could be easily hydrolyzed by peptidases, was replaced with ureido (7), and tested their stabilities in the intestinal juice of rats (Table IV). These studies showed that removal (**5a-b**) and replacement with carboxamide (**6a**) of a carboxyl moiety at the C-terminal are effective, while replacement of the amide moiety with an ureido bond (**7**) showed little effect. These results suggested that the decomposition of FK633 derivatives in intestinal juice may be due to peptidases which recognize the carboxyl moiety at the C-terminal and hydrolyze the amide bond at position B.

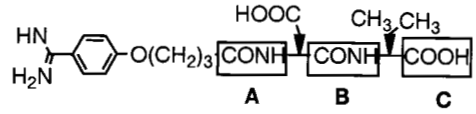


Fig. 8. Putative site (A, B and C for hydrolysis of FK633 in intestinal juice of rats.

Synthesis and evaluation of FR158999 derivatives

Based on the above results, we synthesized a number of orally active derivatives and tested their *in vitro* activities using human platelets (Table V). All showed potent inhibitory activity ($IC_{50} \leq 1$ μM). Among them, two compounds possessing either a morpholine (**6e**) or a thiomorpholine 1,1-dioxide (**6f**, FR 158999) moiety at the C-terminal exhibited potent antiplatelet activity which was 2-3 times more potent than FK633.

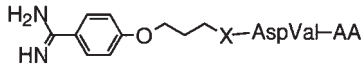
We assessed the *ex vivo* activities of these two compounds in dogs, rats and guinea pigs to evaluate bioavailability (Table VI). Surprisingly, both compounds showed potent *ex vivo* activity after oral administration in all three species, *i.e.*, they were effective at 3.2 mg/kg p.o. in dogs and rats, while FK633 showed only weak antiplatelet activity even at a dose of 20 mg/kg in rats and at 32 mg/kg in dogs. The effect of these two compounds in guinea pigs was similar to that of FK633.

This potent *ex vivo* activity in three different species indicated that the novel peptidic fibrinogen inhibitors, **6e** and FR158999 (**6f**), are orally active and could be clinically useful for treating chronic diseases. We selected FR158999 for further evaluation as a successor to FK633 since the *ex vivo* activity in dogs was slightly more potent than that of **6e**. These results also supported our hypothesis that the weak activity of FK633 in rats and dogs after oral administration was due to hydrolysis by peptidase in the intestinal luminal.

Conclusions

We have described the design, synthesis and evaluation of novel fibrinogen inhibitors. The lead compound in this study was rationally designed from a putative active structure of the RGD peptide using computer simulations. Optimization studies on the C-terminal moiety of the lead

Table IV: Stability of FK633 derivatives in intestinal juice of rats.



Cmpd.	X	AA	Residual (%) (n = 1) 0h	2h	5h	7h
FK633	CO	Val-OH	100	61.3	25.6	19.2
5a	CO	$NHCH_2CH(CH_3)_2$	100	80.4	83.6	86.2
5b	CO	$NHCH_2CH_2Ph(4\text{-}OMe)$	100	100.5	105.7	NT[a]
6a	CO	$Val\text{-}NH_2$	100	105.6	107.3	NT
7	NHCO	Val-OH	100	63.8	45.1	NT

[a]NT= not tested.

Table V: In vitro *antiplatelet activity of compounds* 5 *and* 6.

	AA[a]	Human, ADP IC_{50} (μM)
FK633	Val-OH	0.10 ± 0.01[b]
5a	$NHCH_2CH(CH_3)_2$	1.2 ± 0.3
5b	$NHCH_2CH_2Ph(4\text{-}OCH_3)$	0.44 ± 0.11
5c	$NHCH(CH_3)CH(CH_3)_2$	1.4 ± 0.5
5d	$NHCH_2CH_2Ph(4\text{-}O\text{-}iPr)$	0.37 ± 0.09
5e	$NHCH_2CH_2Ph(4\text{-}OEt)$	0.34 ± 0.12
5f	$NHCH_2CH_2Ph(3,4\text{-}diOCH_3)$	0.54 ± 0.18
6a	$Val\text{-}NH_2$	0.67 ± 0.20
6b	$Val\text{-}NH\text{-}cC_6H_{11}$	0.21 ± 0.10
6c	$Val\text{-}NEt_2$	0.25 ± 0.08
6d	Val-N NCH_3	0.18 ± 0.06
6e	Val-N O	0.079 ± 0.020
6f (FR158999)	Val-N SO_2	0.030 ± 0.010

[a]See Fig.1, in which m=3 for all derivatives; [b]mean ± SD.

compound resulted in the synthesis of our clinical compound (FK633, **3g**) which suppressed *ex vivo* ADP-induced platelet aggregation (> 40% inhibition) and thrombus formation at stented and injured coronary artery after bolus injection at 0.1 mg/kg in dogs, without prolongation of template bleeding time.

We then carried out metabolic studies on FK633 and derivatives to find orally active derivatives. Our research resulted in the discovery of FR158999 (**6f**), a successor to FK633 which is orally bioavailable.

QSAR studies were also performed on FK633 and its derivatives, modified only at the C-terminal moiety. We used logPmw, a novel QSAR parameter for membrane affinity, CLOGP and STERIMOL parameters to elucidate the role of the C-terminal moiety, since few studies on moieties other than the RGD moiety have been carried out even though it is known that these moieties, especially the C-terminal moiety (AA in this study), significantly affect their inhibitory activity. Our QSAR studies showed that logPmw has a good relationship with antiplatelet activity, while CLOGP and STERIMOL parameters have poor relationships, indicating that the important feature of the C-terminal moiety for inhibition is not molecular shape (STERIMOL) or contribution to the compound's total hydrophobicity (CLOGP), but rather its membrane affinity (logPmw). Based on these results, we proposed an inhibition mechanism for these compounds in which active inhibitors bind to the receptor via the membrane (route B in Fig. 6).

Acknowledgements

The authors thank Dr. Takao Takaya and Dr. Keizo Yoshida for their magnanimous support of this study, Dr. Isao Nakanishi for his kind help in performing computer simulations and calculating CLOGP values, Ms. Kayoko Senzaki for carrying out the bioassay, and Dr. David Barrett for preparing the manuscript.

Table VI: Ex vivo activities of ***6e*** *and FR158999 after oral administration in dogs, rats and guinea pigs.*

	Dogs					Rats				Guinea pigs			
	Dose, p.o.	Inhibition (%)				Dose, p.o.	Inhibition (%)			Dose, p.o.	Inhibition (%)		
	(mg/kg)	1	3	6	8h	(mg/kg)	0.5	1	2h	(mg/kg)	1	2	3h
6e	3.2	53 ± 28[a]	45 ± 13	20 ± 11	NT	3.2	95 ± 5	97 ± 2	95 ± 5	NT[b]			
	10	96 ± 32	75 ± 25	59 ± 17	NT								
FR158999	1.0	40 ± 8	11 ± 15	NT	NT	3.2	97 ± 13	97 ± 3	44 ± 14	0.1	49 ± 23	NT	NT
	3.2	85 ± 4	65 ± 8	46 ± 7	0 ± 11					0.32	69 ± 19	50 ± 13	NT
	10	NT	80 ± 4	65 ± 2	45 ± 16					1.0	100 ± 5	59 ± 28	21 ±19[b]

[a]Mean ± SD; [b]NT= not tested.

References and Notes

1. Davies, M.J., Thomas, A.C. *Plaque fissuring - The cause of acute myocardial infarction, sudden ischemic death, and crescendo angina*. Br Heart J 1985, 53: 363-73.

2. Hamm, C.W., Bleifield, W., Kupper, W. et al. *Biochemical evidence of platelet activation in patients with persistent unstable angina*. J Am Coll Cardiol 1987, 10: 998-1004.

3. Plow, E.F., Pierschbacher, M.D., Rouslahti, E. et al. *The effect of Arg-Gly-Asp-containing peptides on fibrinogen and von Willbrand factor binding to platelets.* Proc Natl Acad Sci USA 1985, 82: 8057-61.

4. Ruoslahti, E., Pierschbacher, M.D. *New perspectives in cell adhesion: RGD and integrins.* Science 1987, 238: 491-7.

5. Kieffer, N., Phillips, D.F. *Platelet membrane glycoproteins: Functions in cellular interactions*. Annu Rev Cell Biol 1990, 6: 329-57.

6. Gold, H.K., Gimple, L., Yasuda, T. et al. *Phase I human trial of the potent anti-platelet agent, 7W3-F(ab')$_2$, a monoclonal antibody to the GPIIb/IIIa receptor.* Circulation 1989, 80 (Suppl. 11): 267.

7. Topol, E.J., Califf, R.M., Weisman, H.F. et al. *Randomized trial of coronary intervention with antibody against platelet IIb/IIIa integrin for reduction of clinical restenosis: Results at six months.* Lancet 1994, 343: 881-6.

8. The EPIC Investigators. *Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty.* New Engl J Med 1994, 330: 956-61.

9. Coller, B.S. *Platelets and thrombolytic therapy.* New Engl J Med 1990, 322: 33-42.

10. a) A similar compound to our lead compound (**2**) has been reported by Alig et. al (10b). Their compound, however, includes Gly of the RGD sequence in its structure while Gly is replaced with a methylene chain in compound **2**. b) Alig, L., Edenhofer, A., Hadvary, P. et al. *Low molecular weight, non-peptide fibrinogen receptor antagonists.* J Med Chem 1992, 35: 4393-407.

11. Cook, N.S., Kottirsch, G., Zerwes, H.-G. *Platelet glycoprotein IIb/IIIa antagonists*. Drugs Fut 1994, 19: 135-59.

12. Zablocki, J.A., Miyzno, M., Garland, R.B. et al. *Potent in vitro and in vivo inhibitors of platelet aggregation based upon the Arg-Gly-Asp-Phe sequence of fibrinogen. A proposal on the nature of the binding interaction between the Arg-guanidine of RGDX mimetics and the platelet GPIIb-IIIa receptor.* J Med Chem 1993, 36: 1811-9.

13. Blackburn, B.K., Gadek, T.R. *Glycoprotein IIb/IIIa antagonists.* Ann Rep Med Chem 1993, 28: 79-88.

14. Tanaka, A., Sakai, H., Ishikawa, T. et al. *Design, synthesis, and evaluation of fibrinogen inhibitors, omega-(p-amidinophenoxy)alkanoylaspartic acid derivatives*. Bioorg Med Chem Lett 1996, 6: 1443-8.

15. Tanaka, A., Sakai, H., Ishikawa, T. et al. *Design, synthesis, and evaluation of orally active fibrinogen inhibitors*. Bioorg Med Chem Lett 1997, 7: 521-6.

16. Tanaka, A., Nakamura, K., Nakanishi, I. et al. *A novel and useful descriptor for hydrophobicity, partition coefficient micellar-water, and its application to a QSAR study of antiplatelet agents.* J Med Chem 1994, 37: 4563-6.

17. Tanaka, A., Fujiwara, H. A *QSAR study of fibrinogen inhibitors, 4-(4-amidinophenoxy)butanoylaspartylvaline (FK633) derivatives, using a novel hydrophobic descriptor (logPmw).* J Med Chem 1996, 39: 5017-20.

18. a) We used an in-house program, FIT (18c), which can superimpose a flexible compound on a target compound while rotating flexible dihedral angles. In the FIT program, the variable torsion angles are optimized to minimize the error function E, which is defined in the equation: $E=Ew_id_i+A$, where d_i is the distance between i-th corresponding atomic pair which is selected for superimposing the molecules, w_i is a weight factor on i-th atomic pair and A is the conformational energy term adopted to avoid unfavorable conformations. The value of A is defined as a Lennard-Jones type nonbonded potential function which is used in the SYBYL default force field (18b): $A=E[k_{ab}x(1.0/L^{12}-2.0/L^6)]$, where k_{ab} is the geometric mean of the k constants associated between atom a and atom b, and L is the distance between the two atoms divided by the sum of their radii. b) Clark, M., Cramer III, R.D., Opdenbosch, N.V. *Validation of the general purpose Tripos 5.2 force field*. J Comput Chem 1989, 10: 982-1012. c) Oyasu, H., Nakanishi, I., Tanaka, A. et al. *Conformational studies on the four stereoisomers of the novel anticholinergic 4-(dimethylaniino)-2-phenyl-2-(2-pyridyl)-pentanamide*. J Comput Aided Mol Des 1995, 9: 171-80.

19. a) A β-turn is usually classified under four types, namely type I, I', II and II'. b) See ref. 21 for amino acids such as i, (i+1) or (i+2)th.

20. Krezel, A.M., Wagner, G., Uimer, S.-J. et al. *Structure of the RGD protein decorsin: Conserved motif and distinct function in leech proteins that affect blood clotting*. Science 1994, 264: 1944-7.

21. a) Wilmot, C.M., Thornton, J.M. *Analysis and prediction of the different types of β-turn in proteins.* J Mol Biol 1988, 203: 221-32. b) Crawford, J.L., Lipscomb, W.N., Schellman, C.G. *The reverse turn as a polypeptide conformation in globular proteins.* Proc Natl Acad Sci USA 1973, 70: 538-42.

22. Samnen, J., Ali, F., Romoff, T. et al. *Development of a small RGD peptide fibrinogen receptor antagonist with potent antiaggregatory activity in vitro.* J Med Chem 1991, 34: 3114-25.

23. See ref. 14 for in vitro bioassay methods and ref. 23a for ex vivo bioassays. a) Tanaka, A., Sakai, H., Motoyama, Y. et al. *Antiplatelet agents based on cyclooxygenase inhibition without ulcerogenesis. Evaluation and synthesis of 4,5-bis(4-methoxyphenyl)-2-substituted-thiazoles.* J Med Chem 1994, 37: 1189-99.

24. Compounds whose m values are not 3 or 4 were also synthesized and showed weak activity in the in vitro assay as expected (data not shown).

25. Aoki, T., Cox, D., Senzaki, K. et al. *The anti-platelet and anti-thrombotic effects of FK633, a peptide-mimetic GPIIb/IIIa antagonist.* Thromb Res 1996, 81: 439-50.

26. Ku, T.W., Ali, F.E., Barton, L.S. et al. *Direct design of a potent non-peptide fibrinogen receptor antagonist based on the structure and conformation of a highly constrained cyclic RGD peptide.* J Am Chem Soc 1993, 115: 8861-2.

27. Kopple, K.D., Baures, P.W., Bean, J.W. et al. *Conformations of Arg-Gly-Asp containing heterodimeric cyclic peptides: Solution and crystal studies.* J Am Chem Soc 1992, 114: 9615-23.

28. Reed, J., Hull, W.E., Lieth, C.-W. et al. *Secondary structure of the Arg-Gly-Asp recognition site in proteins involved in cell-surface adhesion*. Eur J Biochem 1988, 178: 141-54.

29. Mickos, H., Bahr, J., Luning, B. *The three-dimensional structure of RGD (Arg-Gly-Asp) adhesion sequence of fibronectin: NMR studies of the peptides RGDS and GRGDSP*. Acta Chem Scand 1990, 44: 161-4.

30. Yamada, T., Song, H, Inaka, K. et al. *Structure of a conformationally constrained Arg-Gly-Asp sequence inserted into human lysozyme*. J Biol Chem 1995, 270: 5687-90.

31. CLOGP values were calculated using Corwin/Leo's CLOGP software version 4.34 by DAYLIGHT Chemical Information Systems, Inc. CLOGP is obtained by calculation to estimate logP, the most common hydrophobicity descriptor, and is widely used in QSAR studies.

32. a) Iwamura, H. *Structure-taste relationship of perillartime and nitro- and cyanoaniline derivatives*. J Med Chem 1980, 23: 308-12. b) Iwamura, H. *Structure-sweetness relationship of L-aspartyl dipeptide analogue. A receptor site topology.* J Med Chem 1981, 24: 572-83.

33. AA moieties of the derivatives were estimated in a QSAR study using STERIMOL parameters because structural differences between compounds (3, 4) mainly arise from differences of the AA moiety.

34. Putative binding pocket of the inhibitors is not in the biomembrane (35a). a) Clues, J.J. *Understanding the structure and function of a prototypic human integrin: The platelet glycoprotein IIb/IIIa complex.* Thromb Haemostas 1994, 72: 1-15.

35. It is thought that the membrane exists in a bilayer condition, consisting of a hydrophilic phase and lipophilic phase. In an aqueous solution of inhibitors, flexible agonists or antagonists of a membrane receptor must construct near to their active conformation; however, it is very rare to construct the near active conformation in solution state. Recently this has become widely accepted in medicinal chemistry so that aqueous lipid (36a), CF_3CH_2OH (36b) or micelle (36c) conditions are often used for determination of active conformation of bioactive and flexible peptides by means of NMR instead of a simple aqueous solution. a) Wakamatsu, K., Okada, T., Miyazawa, T. et al. *Membrane-bound conformation of mastoparan-X, a G-protein-activating peptide*. Biochemistry 1992, 31: 5654-60. b) Doi, M., Kobayashi, Y, Kyogoku, Y. et al. *Structure study of human calcitonin*. Peptides: Chemistry, Structure & Biology, 11th Proc Am Pept Symp (July 9-14, La Jolla, CA). River, J.E., Marshall, G.R. Eds. ESCOM: Leiden 1989, 165-7. c) Motta, A., Pastore, A., Goud, N.A. et al. *Solution conformation of salmon calcitonin in sodium dodecyl sulfate micelles as determined by two-dimensional NMR and distance geometry calculations.* Biochemistry 1991, 30: 10444-50.

36. The activity of FK633 in rats at the dosage of 50 mg/kg after oral administration was increased 3-fold by coadministration of the known peptidase inhibitor, bacitracin (37) (32 mg/kg, data not shown).

37. Storm, D.R. *Mechanism of bacitracin action: A specific lipid-peptide interaction.* Ann NY Acad Sci 1974, 235: 387-98.

38. Decomposition of FK633 by intestinal juice of rats gave a compound whose Val moiety was removed, which was confirmed by HPLC analysis (data not shown).