PLATELET ADHESION and aggregation play important roles in hemostasis and thrombosis. Physiologically, platelets arrest bleeding from damaged blood vessels by sealing wound and initiating the repairing processes. Thrombin-mediated platelet activation and aggregation is partially related to glycoprotein Ib (GPIb) and this membrane glycoprotein also plays a role in regulating the interactions of platelets with their environment via linking with membrane-associated cytoskeleton. Additionally, the interaction of platelet GPIb with von Willebrand factor (vWF) is an important event, allowing platelet adhesion, aggregation, and subsequent thrombus formation in the vessels either with high shear rates or with damaged endothelium. The role of GPIb in the adhesion of platelets to endothelial cell matrix has been studied with monoclonal antibodies (MoAbs). An MoAb against GPIb inhibited the platelet adhesion to endothelial cell matrix, whereas an MoAb against vWF which inhibits the interaction of vWF with platelets has a less pronounced effect, indicating that GPIb has another role in platelet adhesion, apart from serving as the binding site for vWF. Many of the currently investigated antithrombotic drugs interfere with the process of platelet aggregation. However, the inhibition of platelet adhesion may be an important and efficient process for preventing the platelet-rich thrombus formation in the damaged vessels with high shear rate. In addition, thrombin is an important stimulator for platelet aggregation under normal physiological and pathological conditions. Thrombin is an important mediator of platelet aggregation in stenosed canine coronary arteries. Therefore, an inhibitor of thrombin and vWF platelet interaction may be used in the prevention of thrombosis. Several snake venom constituents affecting blood coagulation and platelet aggregation, especially Arg-Gly-Asp (RGD)-containing peptides, have been extensively studied. In addition, GPIb agonists as well as GPIb antagonists have been isolated and characterized. We report here the purification of a protein, crotalin, from Crotalus atrox venom that specifically inhibits ristocetin-induced agglutination or aggregation without affecting the aggregation induced by adenosine diphosphate (ADP) or collagen. Of particular interest is that crotalin, when administered intravenously (IV), markedly delays platelet-rich thrombus formation of the irradiated mesenteric venules in a fluorescein sodium-treated mice model. Among the antithrombotic agents tested, crotalin appears not only to be the most efficacious agent in prolonging the time lapse for inducing platelet-rich thrombus formation in this model, but also exhibits this antiplatelet activity with a longer duration.

MATERIALS AND METHODS

Materials. C. atrox venom was obtained from LATOXAN (Rosans, France). All of DEAE-Sephadex A-50, Sephadex G-75, Mono-S, and Superose columns were purchased from Pharmacia (Uppsala, Sweden). Halsyn was purified from Agkistrodon halys venom as previously described. ADP, U46619, collagen (type I, bovine achilles tendon), and fluorescein sodium were purchased from Sigma Chemical Co (St Louis, MO).

MoAb AP1 raised against platelet GPIb was generously provided by Dr Robert Montgomery (Blood Center of Southeastern Wisconsin Milwaukee) and 7E3, an MoAb raised against platelet GPIIb/IIIa complex, was supplied from Dr Barry Coller (The Mount Sinai Medical Center, New York, NY).

Antithrombotic Effect of Crotalin, a Platelet Membrane Glycoprotein Ib Antagonist From Venom of Crotalus atrox

By Mei-Chi Chang, Hui-Kuan Lin, Hui-Chin Peng, and Tur-Fu Huang

A potent platelet glycoprotein Ib (GPIb) antagonist, crotalin, with a molecular weight of 30 kD was purified from the snake venom of Crotalus atrox. Crotalin specifically and dose dependently inhibited aggregation of human washed platelets induced by ristocetin with IC50 of 2.4 μg/mL (83 nmol/L). It was also active in inhibiting ristocetin-induced platelet aggregation of platelet-rich plasma (IC50, 6.3 μg/mL). Crotalin bound to human platelets in a saturable and dose-dependent manner with a kD value of 3.2 ± 0.1 x 10^-7 mol/L, and its binding site was estimated to be 58,632 ± 3,152 per platelet. Its binding was specifically inhibited by a monoclonal antibody, AP1 raised against platelet GPIb. Crotalin significantly prolonged the latent period in triggering platelet aggregation caused by low concentration of thrombin (0.03 U/mL), and inhibited thromboxane B2 formation of platelets stimulated either by ristocetin plus von Willebrand factor (vWF), or by thrombin (0.03 U/mL). When crotalin was intravenously (IV) administered to mice at 100 to 300 μg/kg, a dose-dependent prolongation on tail bleeding time was observed. The duration of crotalin in prolonging tail bleeding time lasted for 4 hours as crotalin was given at 300 μg/kg. In addition, its in vivo antithrombotic activity was evidenced by prolonging the latent period in inducing platelet-rich thrombus formation by irradiating the mesenteric venules of the fluorescein sodium-treated mice. When administered IV at 100 to 300 μg/kg, crotalin dose dependently prolonged the time lapse in inducing platelet-rich thrombus formation. In conclusion, crotalin specifically inhibited vWF-induced platelet agglutination in the presence of ristocetin because crotalin selectively bound to platelet surface receptor-glycoprotein Ib, resulting in the blockade of the interaction of vWF with platelet membrane GPIb. In addition, crotalin is a potent antithrombotic agent because it pronouncedly blocked platelet plug formation in vivo.

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**INTERACTION OF CROTALIN WITH GPIb**

**Purification of vWF.** According to the modified method of a previous report, vWF was purified from human plasma through cryoprecipitation, polyethylene glycol (PEG) sedimentation, and gel filtration with a Sepharose CL-4B column, and the purity of vWF was assessed by 5% sodium dodecyl sulfate (SDS)-gel electrophoresis.

**Human platelet aggregation.** Blood was collected from healthy human volunteers, who did not take any medication within the 2 weeks before the study, and anticoagulated with 3.8% sodium citrate (9:1, vol/vol). Citrated blood was immediately centrifuged for 10 minutes at 120g and 25°C, and the supernatant (platelet-rich plasma) was obtained. Human washed platelet suspension was prepared as previously described. Washed platelets were suspended in modified Tyrodes’ solution (pH 7.3) (in mmol/L: NaCl, 136.9; CaCl2, 2; KCl, 2.7; MgCl2, 1.0; NaH2PO4, 0.4; NaHCO3, 11.9; glucose, 11.1) containing bovine serum albumin (3.5 mg/mL) and adjusted to about 3 x 108 platelets/mL.

The turbidimetric method, using a Lumi-Aggregometer (Chrono-Log, Havertown, PA), was used to measure platelet aggregation. The extent of aggregation was expressed in light transmission unit.

**Platelet-rich thrombosis model in mice.** According to the modified method of an animal model has been described in detail. An external jugular vein was cannulated for the bleeding time.

**Assay of thromboxane B2 formation.** EDTA (2 mmol/L) and indomethacin (50 µmol/L) were added to platelet suspension at 6 minutes after the addition of thrombin (0.03 U/mL). After centrifugation with an Eppendorf Centrifuge (Model 5414; Hamburg, Germany) at 14,000 cpm for 2 minutes, thromboxane B2 level of the supernatant was determined by thromboxane B2 EIA Kit (Amersham, Buckinghamshire, UK).

**Radiolabeling of crotalin using enzymobead.** The vial of enzymobead reagent (Bio-Rad, San Francisco, CA) was hydrated with 50 µL distilled water at 4°C for 1 hour, followed by adding 50 Ł phosphate buffer, 50 µg crotalin, 0.5 mCi Na125I, and 25 µL β-D-glucose (1%). The mixture was incubated at room temperature for 25 minutes. 125I-conjugated crotalin was eluted through a Sephadex G-10 column at room temperature around microscope was kept at 37°C. The turbidimetric method with an Eppendorf Centrifuge (Model 5414; Hamburg, Germany) was used to measure platelet aggregation. The extent of aggregation was expressed in light transmission unit.

**Platelet aggregation with IC50 value of 6.3 µg/mL (Fig 2), indicating that it might have an antithrombotic effect in vivo.**

**Statistics.** All data are presented as mean ± SEM (n). Student’s t-test was used to assess the statistical differences.

**RESULTS**

**Purification and physicochemical characterization of crotalin.** Crotalin was purified from C.atrox venom through columns of DEAE-Sephadex A-50 and Sephadex G-75 and refractionated by fast protein liquid chromatography using Mono-S (Fig 1A) and Superose columns (Fig 1B). The purified fraction migrated as a single band and the apparent molecular weight was estimated to be 30 kD under nonreducing or reducing conditions by SDS-polyacrylamide (15%) gel electrophoresis (Fig 1B, inset) and named crotalin.

**Amino acid analysis showed that crotalin is a polypeptide, consisting of about 260 amino acid residues (Asp/Asn 36, Glu/Gln 23, Ser 14, Gly 21, His 9, Thr 8, Ala 14, Arg 22, Pro 10, Tyr 6, Val 16, Ile 18, Leu 28, Cys 7, Phe 10, and Lys 16).** The MoAb AP1 (40 to 80 µg/mL)-induced human washed platelet aggregation with IC50 showed a marked inhibitory effect on ristocetin (1.0 mg/mL)-induced human washed platelet aggregation with IC50 of 2.4 µg/mL. This inhibitory effect was independent of the incubation time of crotalin with platelets. Furthermore, crotalin was specific for ristocetin-induced platelet aggregation because it had little effect on the platelet aggregations caused by ADP (20 µmol/L) plus 200 µg/mL of fibrinogen, U46619 (1 µmol/L), or collagen (10 µg/mL). Crotalin also inhibited ristocetin (1 mg/mL)-induced platelet aggregation of platelet-rich plasma with IC50 value of 6.3 µg/mL. This inhibitory effect might have an antithrombotic effect in vivo. Similarly, crotalin (10 µg/mL) apparently did not affect platelet aggregation caused by high concentration of thrombin (>0.05 U/mL). However, crotalin (20 to 100 µg/mL) prolonged the latent period in triggering platelet aggregation in a dose-dependent manner, with a slight inhibition on the maximal aggregation (<30% inhibition). The MoAb AP1 (40 to 80 µg/mL) showed a similar effect to that of crotalin (data not shown).

**Effect of crotalin on thromboxane B2 formation of platelets caused by thrombin and other agonists.** As shown in Table 1, crotalin at a lower concentration (5 to 10 µg/mL) inhibited thromboxane B2 formation of platelets stimulated by ristocetin and vWF. At higher concentration (20 µg/mL), crotalin significantly inhibited thromboxane B2 formation of platelets caused by-thrombin.
by thrombin (0.03 U/mL), whereas it did not affect thrombox-
ane B2 formation stimulated by collagen.

Characterization of the binding of 125I-crotalin to human
platelets. 125I-crotalin bound to platelets in a dose-dependent
manner, reaching a saturated binding at 10 µg/mL (0.33 µmol/L)
(Fig 3). The Scatchard analysis of 125I-crotalin binding data
showed that the binding sites of crotalin were 58,632 ± 3,152
per platelet with a kd value of 3.2 ± 0.1 × 10⁻⁷ mol/L (Fig 4).
125I-crotalin binding to platelets was blocked either by unla-
beled crotalin (100 µg/mL, >85%) or AP1 (20 µg/mL, >90%),
but not by the MoAb against GPIIb/IIIa, 7E3 (40 µg/mL), or 5
mmol/L EDTA (data not shown).

Effect of crotalin on bleeding time of mice. IV administra-
tion of crotalin to mice significantly prolonged the bleeding
time in a dose-dependent manner (Fig 5). Crotalin pro-
nouncedly prolonged the bleeding time (>10 minutes) as
measured 1 hour after a bolus injection of crotalin (300 µg/kg),
and this effect slowly ran down thereafter (Fig 6). The bleeding
time almost returned to the baseline level within 4 hours after
the administration of crotalin. However, the platelet count, as
measured at 10 minutes after the administration of crotalin (300 µg/kg), did not show a major change, although about 20% decrease was observed (128 ± 10 × 10^4, n = 4, control v 104 ± 13 × 10^4 platelets/µL, n = 4, experimental; P > .05). Even when the dose of crotalin was increased to 600 µg/kg, a transient slight decrease of platelet count (about 20%) was observed at the first 5 minutes after the administration of crotalin, and the platelet count returned to control level within 20 minutes.

**Platelet-rich thrombus formation in the microvessels.** As the given dose of fluorescein sodium was increased, the latent period in inducing platelet plug formation was shortened (Table 2). IV administration of crotalin at 300 µg/kg pronouncedly delayed platelet-rich thrombus formation and significantly prolonged the occlusion time in mice receiving different doses of fluorescein sodium (Table 2). The occlusion time was lengthened from 100 ± 14 to 311 ± 25 seconds in fluorescein sodium (150 µg per mouse)-treated mice after IV administration of crotalin. Crotalin dose-dependently prolonged the occlusion time in causing platelet plug formation, and administration of 300 µg/kg of crotalin resulted in the maximal lengthening of occlusion time (Table 3). This antithrombotic effect lasted at least for 2 hours (Fig 7). On the other hand, a continuous infusion of prostaglandin I₂ (PGI₂) at 0.5 µg/kg/min, as in our previous study, 29 showed a maximal lengthening effect on occlusion time. Higher dose (2 µg/kg/min) of PGI₂ did not

**Fig 2.** Dose-response relationship of crotalin on platelet aggregation induced by 1 mg/mL of ristocetin in human washed platelets in the presence of 10 µg/mL of vWF (●) or in platelet-rich plasma (▲). Values are presented as mean ± SEM (n = 4).

**Fig 3.** Binding isotherm of ³²P-crotalin on human platelet suspension. Platelets were incubated with various concentrations of ³²P-crotalin. Total binding (●) and nonspecific binding (■) in the presence of unlabeled crotalin (200 µg/mL) were determined, respectively. Specific binding (■) was calculated by subtracting the nonspecific binding from total binding. This is a representative one of four similar experiments.

**Fig 4.** Scatchard plot of the ³²P-crotalin binding to human washed platelets. This plot is a representative one of four experiments.

**Table 1. Effects of Crotalin on Thromboxane B₂ Formation of Washed Platelets Stimulated by Thrombin and Other Agonist**

<table>
<thead>
<tr>
<th></th>
<th>Thromboxane B₂ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ristocetin + vWF</td>
</tr>
<tr>
<td>Control</td>
<td>12.4 ± 2.6</td>
</tr>
<tr>
<td>Crotalin</td>
<td></td>
</tr>
<tr>
<td>5 µg/mL</td>
<td>3.4 ± 0.6*</td>
</tr>
<tr>
<td>10 µg/mL</td>
<td>3.5 ± 0.4*</td>
</tr>
<tr>
<td>20 µg/mL</td>
<td>ND</td>
</tr>
<tr>
<td>40 µg/mL</td>
<td>ND</td>
</tr>
</tbody>
</table>

Human washed platelets were preincubated with saline or various concentrations of crotalin at 37°C for 5 minutes before the addition of ristocetin (1 mg/mL) plus vWF (10 µg/mL), thrombin (0.03 U/mL), and collagen (10 µg/mL). Six minutes after stimulation, the reaction was terminated by the addition of EDTA (2 mmol/L) and indomethacin (50 µmol/L). The basal value of resting platelets was 2.4 ± 0.5 ng/mL. Values are presented as mean ± SEM.

Abbreviation: ND, not determined.

* P < .01 as compared with the respective control, n = 5.
† P < .05.
further increase its antithrombotic activity. Halysin, an RGD-containing peptide purified from venom of A. halys, inhibited platelet aggregation via a competitive inhibition of fibrinogen binding to platelet GPIIb/IIIa.\textsuperscript{30} It completely inhibited ex vivo platelet aggregation of platelet-rich plasma induced by collagen (15 µg/mL) 20 minutes after the IV administration of halysin at the dose of 10 mg/kg (data not shown). In comparing the maximal effect of PGI\textsubscript{2}, halysin, ancrod,\textsuperscript{29} and crotalin on the occlusion time in the same in vivo model, crotalin appears to be the most efficacious agent in prolonging the occlusion time (Table 4).

**DISCUSSION**

Crotalin, a newly purified protein from the venom of C. atrox, specifically inhibited ristocetin-induced platelet aggregation in vitro and exhibited the antithrombotic activity in vivo. Crotalin specifically inhibited platelet aggregation induced by ristocetin either in platelet suspension supplemented with vWF or in platelet-rich plasma with an IC\textsubscript{50} of 2.4 and 6.3 µg/mL, respectively. In contrast to RGD-containing peptides, crotalin at 40 µg/mL did not affect collagen- and U46619-induced platelet aggregation. Ristocetin-induced platelet aggregation was mediated through the initial binding of vWF to platelet GPIb, and subsequently resulted in the exposure of the fibrinogen receptor.\textsuperscript{31,32} The 125\textsuperscript{I}-crotalin binding site was 58,632 per platelet with a kd value of 3.2 3 10\textsuperscript{-7} mol/L. 125\textsuperscript{I}-crotalin binding to platelets was selectively inhibited by AP1, an MoAb raised against platelet GPIb, but not by 7E3, an MoAb raised against GPIIb/IIIa. The binding was not affected by EDTA, indicating that the binding process is divalent-cation independent. Through the analysis of binding data and its inhibitory activity on ristocetin-induced platelet aggregation, crotalin appears to be a selective antagonist of platelet membrane GPIb. Regarding the binding sites of crotalin, the estimation of 58,632 per platelet is higher than that estimated with GPIb MoAb (around 30,000). It has been reported that the binding sites of other venom GPIb

| Table 2. Effect of Fluorescein Sodium and Crotalin on the Occlusion Time in Causing Platelet-Rich Thrombus Formation in Mesenteric Venules of Mice |
| Dose of Fluorescein Sodium per Mouse | 100 µg | 150 µg | 200 µg |
| Normal saline | 180 ± 23 (4) | 100 ± 14 (4) | 63 ± 7 (3) |
| Crotalin (300 µg/kg) | 310 ± 36 (4)* | 311 ± 25 (5)* | 155 ± 13 (3)* |

Values are expressed as elapsed time (in seconds) in causing platelet plug formation on the irradiation of venules and presented as mean ± SEM (n).

*\(P < .001\) as compared with the respective control.

(15 µg/mL) 20 minutes after the IV administration of halysin at the dose of 10 mg/kg (data not shown). In comparing the maximal effect of PGI\textsubscript{2}, halysin, ancrod,\textsuperscript{29} and crotalin on the occlusion time in the same in vivo model, crotalin appears to be the most efficacious agent in prolonging the occlusion time (Table 4).

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**Table 3. Dose-Response Relationship of Crotalin on the Elapsed Time of Inducing Thrombus Formation on Light Irradiation of Venules of Mice Pretreated With Fluorescein Sodium (150 µg per mouse)**

<table>
<thead>
<tr>
<th>Dose of Crotalin (µg/kg)</th>
<th>Occlusion Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Crotalin</td>
</tr>
<tr>
<td>100</td>
<td>94 ± 7 (4)</td>
</tr>
<tr>
<td>200</td>
<td>97 ± 20 (4)</td>
</tr>
<tr>
<td>300</td>
<td>99 ± 19 (4)</td>
</tr>
</tbody>
</table>

The occlusion time was expressed (in seconds) as the time lapse for inducing platelet-rich thrombus formation on the irradiation of the venules in fluorescein sodium-treated mice. The significant difference of elapsed time was compared between groups before and after administration of crotalin. Values are presented as mean ± SEM (n).

*\(P < .05\)

†\(P < .001\)
INTERACTION OF CROTALIN WITH GPIb

Fig 7. The effects of crotalin on the elapsed time in causing platelet plug formation upon the irradiation of venules in mice. Fluorescein sodium (150 µg per mouse) was intravenously injected 10 minutes before the irradiation, and the irradiation was then started for inducing the formation of thrombus at the indicated time intervals after the IV administration of crotalin (300 µg/kg). Values are presented as mean ± SEM (n = 5-6). BL indicates baseline value. (∨) Represents the occlusion time of each mouse measured at the indicated time. *P < .05, **P < .01 as compared with basal value.

Platelet-rich thrombus formation was induced as described in Table 2. The occlusion time was measured 10 to 20 minutes after the administration of halysin, ancrod, crotalin, and PGi2. Values are presented as mean ± SEM (n = 6).

Saline 105 ± 12 (6)
PGi2, 2 µg/kg/min 148 ± 16 (6)*†
Halysin, 10 mg/kg 155 ± 20 (6)*†
Ancrod, 1 U/kg 176 ± 31 (6)*†
Crotalin, 300 µg/kg 293 ± 38 (6)*

Table 4. Effect of Crotalin, PGi2, Halysin and Ancrod on the Lapsed Time in Inducing Thrombus Formation Caused by Irradiation of Mesenteric Venules of Fluorescein Sodium-Treated Mice

Platelet-rich thrombus formation was induced as described in Table 2. The occlusion time was measured 10 to 20 minutes after the administration of halysin, ancrod, crotalin, and PGi2. Values are presented as mean ± SEM (n = 6).

*P < .05 as compared with the control group (receiving normal saline).
†P < .05 as compared with crotalin group.

In this study, crotalin as well as AP1 prolonged the latent period in triggering platelet aggregation caused by low concentrations of thrombin (0.03 U/mL), with a slight inhibition on platelet aggregation. Crotalin blocked thromboxane B2 formation of platelets challenged by ristocetin plus vWF or low concentration of thrombin, confirming the hypothesis that the ligation of GPIb may lead to the activation of endogenous phospholipase A2. Regarding the molecular characterization of crotalin, a platelet GPIb antagonist, the preliminary data show that crotalin is unique as a single chain polypeptide that is quite different from the known venom GPIb-binding proteins because they are heterodimer in nature, sharing a highly homologous sequence with C-type lectins. Whether it exists as a monomer or dimer under physiological condition is unknown. However, the detailed characterization of the physicochemical properties of crotalin is in progress.

An IV infusion of crotalin lengthened bleeding time of mice in a dose-dependent manner. Maximal prolongation was observed during a period of 10 to 60 minutes after injection of crotalin (300 µg/kg), and bleeding time progressively returned to control values over a 4-hour period. However, the platelet count after the administration of crotalin did not show a major change. It has been reported that echinatine and jararacussu GPIb-BP caused a transient thrombocytopenia in mice, and therefore it may be an advantage of crotalin when considering its potential use as antithrombotic agent. From its in vivo experiment, we suggest that crotalin may inhibit platelet aggregation as well as platelet adhesion to subendothelium in vivo through blocking the interaction of vWF with platelet GPIb.

As the first step in hemostasis or thrombosis, the binding of vWF to platelet GPIb is essential for platelet adhesion at high-shear blood flow. The platelets from patients with Bernard-Soulier Syndrome were defective in expression of functional GPIb-V-IX complex, and poorly adhered to subendothelium at all shear rates. Much effort has recently been devoted to characterize the interaction of vWF and platelet GPIb at the molecular level with an aim of developing inhibitors that could be useful in the prevention of thrombosis. Recently, it has been indicated that inhibition of vWF-platelet GPIb interaction is effective in preventing acute restenosis after thrombolytic therapy.

In the present study we evaluated the antithrombotic effect of crotalin in a mouse model. Surprisingly, crotalin apparently delayed platelet-rich thrombus formation in mesenteric microvessels and its antithrombotic activity was dose dependent. The time course of its antithrombotic effect was consistent with that of its effect on bleeding time in mice (Figs 6 and 7). Table 4 shows the minimal dose of PGi2, halysin, ancrod, and crotalin in causing the maximal prolongation of occlusion time in this platelet-rich thrombus animal model. Halysin, an RGD-containing venom peptide, completely inhibited ex vivo platelet aggregation for 20 minutes after the administration of halysin at 10 mg/kg. Ancrod (1 U/kg), a thrombin-like enzyme, caused defibrinogenation and exhibited antiplatelet activity for 60 minutes. Of the compounds tested, crotalin showed the most pronounced effect in prolonging the occlusion time of the irradiated vessels in inducing platelet-rich thrombus formation as compared with PGi2, halysin, and ancrod, indicating that blockade of the interaction between vWF and platelet GPIb may be a potential strategy in causing a marked antithrombotic
effect. In addition, crotalin exhibits an antithrombotic activity with a longer duration as compared with short duration of PGI2, halysin or another disintegrin, triflavin.40

Considering the pharmacokinetic of crotalin, crotalin may be more active as an antithrombotic agent in mice than in human beings because the effective dosage of crotalin in mice ranged from 100 to 300 µg/kg, equivalent to 1.3 to 3.8 µg/mL (assuming 2.0 mL plasma per mouse), even if protein binding is neglected. However, the IC50 of crotalin was about 6.3 µg/mL (in human platelet-rich plasma), five times higher than the effective dosage of 100 µg/kg in mice. On the other hand, the in vivo antithrombotic effect of crotalin in mice may result from both the antiplatelet and anticoagulant activities because the preliminary results show that crotalin prolonged the whole blood clotting time and activated partial thromboplastin time but not the prothrombin time as crotalin was administered IV (unpublished data, December 1996). However, its anticoagulant activity is under investigation.

In conclusion, crotalin specifically inhibited ristocetin-induced platelet agglutination in the presence of vWF through a selective binding of platelet membrane GPIb, resulting in a blockade of interaction between vWF and GPIb. Furthermore, crotalin markedly prolonged the bleeding time when administered IV into mice and was efficacious in blocking platelet plug formation in vivo experimental model. Therefore, crotalin may be a valuable tool for developing a new class of antithrombotic drugs for clinic use through the study of its structure-activity relationship.

ACKNOWLEDGMENT

We appreciate the secretarial work of I.S. Peng.

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