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Effect of a humanized monoclonal antibody to von Willebrand factor in a canine model of coronary arterial thrombosis

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Abstract

The purpose of this study was to investigate the antithrombotic effect and bleeding time prolongation of AJW200, a humanized monoclonal antibody to von Willebrand factor (vWF), in a canine model of coronary arterial thrombosis. AJW200 significantly inhibited cyclic flow reductions, as well as botrocetin-induced platelet aggregation, at 0.1 mg/kg. A significant prolongation of bleeding time was observed at 0.3–1 mg/kg. Approximately 50% occupancy of vWF (approximately 0.7 μg/ml AJW200 in plasma) and 80–100% occupancy (approximately 20 μg/ml AJW200 in plasma) were needed for the antithrombotic effect and the extensive prolongation of bleeding time, respectively. On the contrary, the minimal effective dose of abciximab (0.8 mg/kg) was associated with a significant prolongation of bleeding time. These results suggest that the pharmacological blockade of platelet glycoprotein (GP) Ib-vWF interaction with AJW200 results in a safer antithrombotic profile than platelet GPIIb/IIIa blockade with abciximab in dogs.

Keywords: Von Willebrand factor (vWF); Platelet; Thrombosis; Bleeding time; AJW200

1. Introduction

Platelet aggregation, which is mediated by the interaction of platelet GPIIb/IIIa and plasma fibrinogen or vWF, plays a central role in the arterial thrombus formation. Previous clinical studies have demonstrated that a chimeric monoclonal antibody Fab against GPIIb/IIIa, abciximab (ReoPro™), improved clinical outcomes in patients with acute coronary syndromes undergoing percutaneous coronary intervention (Topol et al., 1994; The EPILOG Investigators, 1997; The EPISTENT Investigators, 1998). Although abciximab has been widely used for the treatment of such patients, the clinically available dose is known to prolong the bleeding time extensively (Tcheng et al., 1994). Also, multivariate analysis revealed that use of abciximab was independently associated with major and minor bleeding (Cote et al., 2001). In addition, the recent clinical trial demonstrated that abciximab was not beneficial in patients with acute coronary syndromes not undergoing percutaneous coronary intervention and that bleeding rates increased with the duration time (The GUSTO IV-ACS Investigators, 2001). Such unfavorable effects might be due to the potential of abciximab to interfere with all types of platelet aggregation.

When the blood runs through the stenosed coronary arteries, the shear stress is extremely elevated (520–3349 or 350 dyn/cm^2 as an average value) in dogs (Strony et al., 1990, 1993). In addition, the previous reports demonstrated that the enhancement of high shear stress-induced platelet aggregation was observed in patients with acute coronary syndromes (Goto et al., 1999; Eto et al., 1999). Especially under high shear stress conditions, platelet GPIb–vWF interaction plays a crucial role in platelet-mediated thrombus formation (Sakariassen et al., 1979; D'Alessio et al., 1990). We, therefore, hypothesized that the pharmacological blockade of GPIb–vWF interaction would be beneficial for the treatment of patients with acute coronary syndromes, and that it shows a lower bleeding risk than the GPIIb/IIIa blockade. Actually, previous studies indicated that the GPIb–vWF blockers, including aurintricarboxylic acid and a peptide fragment derived from vWF, inhibited coronary arterial thrombosis in dogs and baboons, respectively (Strony et al., 1990; McGhie et al., 1994). However, none of the tested agents have been proven effective in clinical applications yet.

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We have demonstrated that AJvW-2 is a murine monoclonal antibody to human vWF, which recognize the A1 domain, was a specific blocker of the GPIb–vWF interaction (Kageyama et al., 1997). In addition, the Fab fragment of AJvW-2 inhibited a repetitive coronary arterial thrombosis in dogs (Kageyama et al., 2001). Although these reports suggested that AJvW-2 could be a new therapeutic agent for treatment of patients with acute coronary syndromes, murine immunoglobulin (Ig) G_1 was expected to show the immunogenicity when administered to humans. Recently, we have succeeded in the humanization of AJvW-2 by grafting of the complementarity determining region, to reduce its immunogenicity. In addition, we converted a constant region of AJvW-2 from IgG_1 to IgG_4, to reduce the potency of unfavorable Fc functions. A humanized AJvW-2 (named AJW200) specifically inhibited vWF-mediated platelet adhesion, aggregation and activation at the comparative concentrations to parent monoclonal antibody (Kageyama et al., 2002). Also, a sustained inhibition of ristocetin-induced platelet aggregation was observed following an intravenous single bolus administration of AJW200 in cynomolgus monkeys (Kageyama et al., 2002). However, the in vivo antithrombotic profile of AJW200 has not been investigated yet.

The purposes of this study were to evaluate the efficacy of AJW200 in a canine model of coronary arterial thrombosis and to compare the therapeutic window with that of abciximab. In addition, we investigated the correlation of vWF occupancy or plasma AJW200 concentration with the antiplatelet, antithrombotic effects and the bleeding time prolongation.

2. Materials and methods

2.1. Animals

Male beagle dogs weighing 10–13 kg were obtained from Narc (Chiba, Japan) and housed in a controlled environment and provided with standard chow and water. The care and use of the dogs were in accordance with the Institutional Animal Care and Use Committee of the Pharmaceutical Research Laboratories of Ajinomoto.

2.2. Surgical preparations

The following experimental procedure was originally described by Folts (1982), Folts et al. (1976) and Bertha and Folts (1984). Briefly, beagle dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), intubated with a cuffed endotracheal tube and ventilated with room air by a mechanical respirator. The cephalic veins in the right and left forelegs were cannulated for the general anesthesia via infusion of sodium pentobarbital (0.1 mg/kg/min) and for the administration of antibody, respectively. The cephalic vein in the left hindleg was also cannulated for the continuous administration of 6% hydroxyethyl starch. The right femoral artery and the left carotid artery were cannulated for the continuous monitoring of blood pressure and left ventricular pressure, respectively. The heart was exposed through a left lateral thoracotomy at the fifth intercostal space and suspended in a pericardial cradle. A 1.5-cm segment of the left circumflex coronary artery was exposed and dissected free from the surrounding tissue. All branches within the dissected segment were ligated. An electromagnetic flow probe (Nihon Kohden) was placed at the proximal portion of dissected vessel for measurement of the phasic and mean coronary blood flow. Electrocardiogram lead II with the heart rate was also measured. These hemodynamic parameters were continuously monitored with a polygraph system (Nihon Kohden) and recorded on an eight-channel recorder (Nihon Kohden).

After 30 min for stabilization, the endothelium of the left circumflex coronary artery was injured by gently squeezing with a hemostat clamp. A hard, plastic cylindrical constrictor was then placed at the injured site of left circumflex coronary artery, to produce a gradual decline in coronary blood flow due to platelet-rich thrombus formation. When blood flow reached zero, blood flow was restored by gently shaking the constrictor to dislodge the platelet aggregates mechanically. This repetitive pattern of decreasing blood flow following mechanically restoration was referred to cyclic flow reductions. Additional endothelial injuries or the appropriate constrictor selection were repeated until stable cyclic flow reductions were observed. After a 60-min control period of reproducible cyclic flow reductions ($t=-60$ to 0 min), antibody was administered via an intravenous bolus injection ($t=0$ min) and coronary blood flow was measured until the following 60 min ($t=+60$ min).

2.3. Study design

Forty of the 56 operated dogs showed the stable cyclic flow reductions and were divided into eight groups ($n=5$ each): control (saline), AJW200 (0.03, 0.1, 0.3 and 1 mg/kg) and abciximab (0.2, 0.4 and 0.8 mg/kg). The antithrombotic effect was evaluated by comparing the postdosing frequency of cyclic flow reductions (0 to +60 min) with predosing one (−60 to 0 min). Both the blood collection and the measurement of bleeding time were performed at −60, +5 and +60 min. The ex vivo platelet aggregation, hematological parameters, as well as coagulation parameters, were also measured. The residual plasma samples were frozen and stored at −80 °C to measure plasma vWF level, vWF occupancy and plasma AJW200 concentration.

2.4. Ex vivo platelet aggregation, hematological and coagulation parameters

Citrated blood samples (final 0.38%) were collected from the right femoral artery via the cannula at each time point. Platelet-rich plasma and platelet-poor plasma were prepared by the centrifugation of blood at 800 rpm for 15 min and at

2700 rpm for 10 min, respectively. Hematological parameters including platelet count were measured with an automated cell counter (Sysmex). The platelet count in platelet-rich plasma was adjusted to 150,000–250,000/μl by diluting with platelet-poor plasma. Platelet aggregation induced by botrocetin (final 0.5 U/ml, American Diagnostica) or ADP (final 20 μM, Meiji Yakuhin) was measured by the change in light transmission (PPP = 100%) in an aggregometer (MCM Hematracer 801, MC Medical). Also, prothrombin time and activated partial thromboplastin time were measured with an automated blood coagulation analyzer (Sysmex) using the residual platelet-poor plasma.

2.5. Bleeding time measurements

The template bleeding time was measured at the surface of the inner upper lip using an automated spring-loaded device (Simplate R, Organon Teknika) at each time point. Until the visual cessation of blood onto the filter paper, the measurement was performed up to 30 min at serial intervals of 30 s.

2.6. Plasma vWF antigen level

The plasma vWF antigen level was measured by a sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well microtiter plates were coated with rabbit antihuman vWF polyclonal antibody (10 μg/ml, Dako) at 4 °C overnight. The plates were blocked with 1% bovine serum albumin in phosphate-buffered saline at room temperature for 1 h. Diluted plasma samples were added and incubated at room temperature for 2 h. After washing, peroxidase-conjugated rabbit antihuman vWF polyclonal antibody (dilution 1:3000, Dako) was incubated at room temperature for 1 h. After washing, bound vWF molecules were quantified by measuring absorbance at 490 nm. The plasma vWF antigen level was calculated by comparing the optical density of each plasma with that of the diluted corresponding predosing plasma and expressed as a percentage of the predosing value.

2.7. vWF occupancy

The rate of the binding sites occupied by AJW200 in plasma vWF was determined by a sandwich ELISA. Briefly, 96-well microtiter plates were coated with goat antihuman vWF antibody (10 μg/ml, Cedarlane lab) at 4 °C overnight. The plates were blocked with 1% bovine serum albumin in phosphate-buffered saline at room temperature for 2 h. Diluted predosing plasma was preincubated with AJW200 (final 3.125–800 ng/ml) as standard samples. After washing, diluted postdosing plasma and standard samples were added and incubated at room temperature for 2 h. After washing, peroxidase-conjugated mouse antihuman IgG_4 monoclonal antibody (dilution 1:5000, southern biotechnology associates) was incubated at room temperature for 1 h. After washing, bound AJW200 was quantified by measuring absorbance at 490 nm. A standard curve was constructed for each animal. The optical density when AJW200 was not added and the saturated maximal optical density were defined as those of 0% and 100% occupancy of vWF, respectively. Relative vWF occupancy of the postdosing was calculated.

2.8. Plasma concentration of AJW200

The plasma concentration of AJW200 (mixture of the free and vWF-bound form) was determined by a sandwich ELISA. Briefly, 96-well microtiter plates were coated with rabbit antihuman vWF polyclonal antibody (10 μg/ml, Dako) at 4 °C overnight and blocked with 1% bovine serum albumin in phosphate-buffered saline at room temperature for 2 h. After washing, diluted postdosing plasma and standard AJW200 solutions (0.78–100 ng/ml) were added and incubated for 2 h at room temperature. Human vWF concentrate was added to each sample at final 0.025 U/ml as a factor VIII:C activity. After washing, peroxidase-conjugated mouse antihuman IgG_4 monoclonal antibody (dilution 1:5000, southern biotechnology associates, Inc.) was added and incubated at room temperature for 1 h. The absorbance was measured at 490 nm and the plasma concentration of AJW200 was calculated from a standard curve.

2.9. Antibodies

AJW200, a humanized AJvW-2, is an IgG_4 humanized monoclonal antibody to human vWF and derived from Sp2/0 mouse myeloma cells. Abciximab (c7E3 Fab) was purchased from Eli Lilly (Indianapolis).

2.10. Statistics

Data are expressed as the means ± S.E.M. ($n = 5$). The frequency of cyclic flow reductions, ex vivo platelet aggregation, hematological parameters, prothrombin time, activated partial thromboplastin time, hemodynamic parameters and plasma vWF antigen level were subjected to repeated-measures analysis of variance (ANOVA) followed by Dunnett test. Bleeding time was subjected to nonparametric Kruskal–Wallis test followed by Dunnett test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cyclic flow reductions and hemodynamics

During the stable cyclic flow reductions, the decreases in blood pressure and left ventricular pressure (especially its first derivation, dP/dt), as well as ST-segment elevation, were transiently observed in accordance with coronary blood flow reaching zero (data not shown). Fig. 1 demonstrates the inhibitory effect of AJW200 on cyclic flow reductions

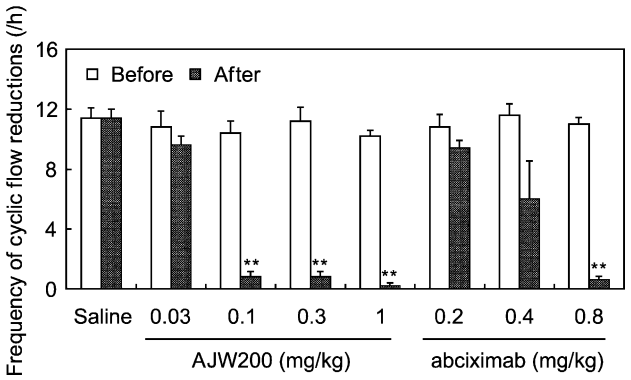

Fig. 1. Effects of AJW200 and abciximab on the frequency of cyclic flow reductions in a canine model of coronary arterial thrombosis. $^{**}P<0.01$ vs. control (saline) group. Data are represented as means ± S.E.M. ($n=5$).

compared to that of abciximab. The frequency of cyclic flow reductions was not affected by injection of saline (from 11.4 ± 0.7 to 11.4 ± 0.6 cycles/h; not significant). AJW200 significantly inhibited cyclic flow reductions at 0.1 mg/kg or above. On the contrary, abciximab significantly inhibited cyclic flow reductions at 0.8 mg/kg. No changes in heart rate, blood pressure and left ventricular pressure were observed between groups (data not shown).

3.2. Bleeding time

The effects of AJW200 and abciximab on the bleeding time are shown in Fig. 2. At baseline (− 60 min), no difference in the bleeding time between groups was observed (panel A). Both AJW200 and abciximab prolonged bleeding time dose-dependently (panels B and C, respectively). The significant prolongation was observed at 1 and 0.8 mg/kg, respectively. The effect of 0.3 mg/kg AJW200 was moderate but significant at 60 min. Furthermore, the extensive prolongation (≥ 30 min) was observed in two of five animals treated with 1 mg/kg AJW200 and two of five animals treated with 0.8 mg/kg abciximab.

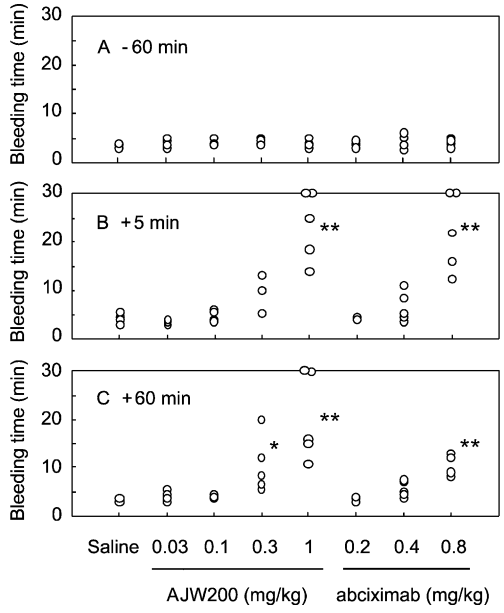

Fig. 2. Effects of AJW200 and abciximab on template bleeding time in dogs. Bleeding times were measured at − 60 min (A), + 5 min (B) and + 60 min (C). $^{*}P<0.05$, $^{**}P<0.01$ vs. control (saline) group.

3.3. Ex vivo platelet aggregation, hematological and coagulation parameters

The effects of AJW200 and abciximab on the ex vivo platelet aggregation are shown in Fig. 3. Botrocetin-induced platelet aggregation was significantly inhibited following administration of AJW200 (≥ 0.1 mg/kg) and the inhibition was sustained during 60 min at all doses (panel A). The inhibitions were 18%, 70%, 81% and 76%, 5 min after the administration of 0.03, 0.1, 0.3 and 1 mg/kg AJW200, respectively. Abciximab did not affect botrocetin-induced platelet aggregation. Whereas ADP-induced platelet aggregation was inhibited following administration of abciximab dose-dependently and the inhibition was time-dependently reduced (panel B). The inhibitions were 29%, 62% and 79%, 5 min after the administration of 0.2, 0.4 and 0.8 mg/kg

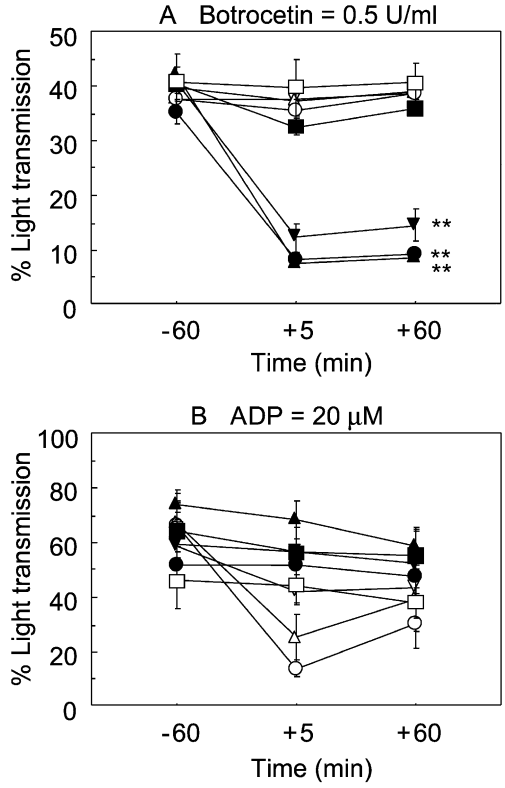

Fig. 3. Effects of AJW200 and abciximab on ex vivo platelet aggregations induced by botrocetin (A) and ADP (B) in dogs. Dogs were treated with saline (□), AJW200 (0.03 mg/kg, ■; 0.1 mg/kg, ▼; 0.3 mg/kg, ▲; 1 mg/kg, ●) and abciximab (0.2 mg/kg, ∞; 0.4 mg/kg, △; 0.8 mg/kg, ○). $^{**}P<0.01$ vs. control (saline) group. Data are represented as means ± S.E.M. ($n=5$).

Table 1
Plasma vWF level, vWF occupancy and plasma AJW200 concentration following administration of AJW200 in dogs

	Dose (mg/kg)			
	0.03	0.1	0.3	1
Plasma vWF level (% of pretreatment value)				
+5 min	98 ± 24	84 ± 10	99 ± 8	98 ± 4
+60 min	97 ± 23	99 ± 3	106 ± 11	105 ± 5
vWF occupancy (%)				
+5 min	21 ± 5	74 ± 9	87 ± 6	98 ± 2
+60 min	20 ± 3	62 ± 7	82 ± 6	89 ± 6
AJW200 concentration (μg/ml)				
+5 min	0.434 ± 0.093	1.273 ± 0.208	5.394 ± 0.51	25.419 ± 0.995
+60 min	0.369 ± 0.048	1.207 ± 0.179	4.366 ± 0.527	24.327 ± 1.202

Data are represented as means ± S.E.M. ($n = 5$).

abciximab, respectively. AJW200 did not affect ADP-induced platelet aggregation.

The positive correlation was observed between the platelet inhibition and the inhibition of cyclic flow reductions ($r^2 = 0.79$ for AJW200; $r^2 = 0.75$ for abciximab). No significant changes in hematological (leukocytes, erythrocytes, hemoglobin, hematocrit, and platelets) and coagulation parameters (prothrombin time and activated partial thromboplastin time) were observed between groups (data not shown).

3.4. Plasma vWF antigen level, vWF occupancy and plasma concentration

The summarized data were shown in Table 1. The plasma vWF antigen level was not affected by any treatment and no significant change was observed between groups. The vWF occupancy, as well as the plasma AJW200 concentration, increased in a dose-dependent fashion, and the saturated occupancy was observed at 0.3–1 mg/kg. In addition, both parameters were maintained for 60 min after the administration of AJW200.

The correlation between the vWF occupancy and the efficacy of AJW200 (panel A) and the bleeding time prolongation (panel B) are shown in Fig. 4. Approximately 50% occupancy of vWF was minimally needed for the inhibition of cyclic flow reductions as well as platelet inhibition. Bleeding time was extensively prolonged, when the vWF occupancy reached 80–100%.

The correlation between the plasma AJW200 concentration and the efficacy of AJW200 (panel A) and the bleeding time prolongation (panel B) are shown in Fig. 5. Approximately 0.7 μg/ml in plasma was minimally needed for the inhibition of cyclic flow reductions as well as platelet inhibition. When AJW200 concentration reached approximately 20 μg/ml in plasma, the bleeding time was extensively prolonged.

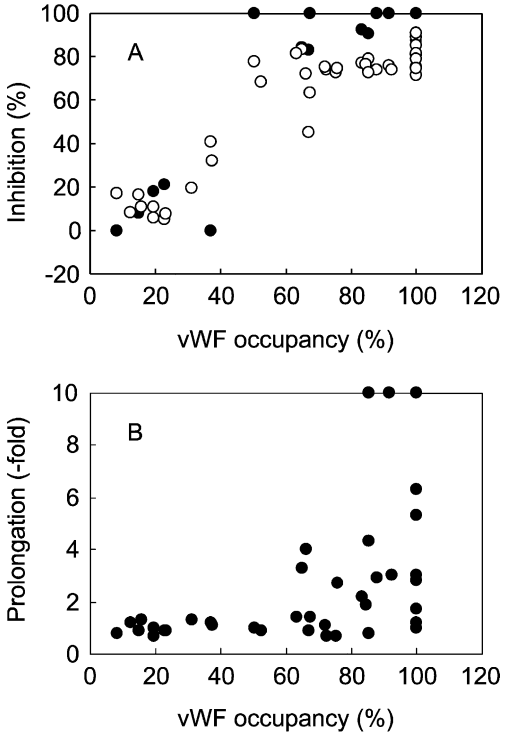

Fig. 4. (A) Correlation of vWF occupancy with the inhibition of ex vivo botrocetin-induced platelet aggregation (○), inhibition of cyclic flow reductions (●). (B) Correlation of vWF occupancy with bleeding time prolongation.

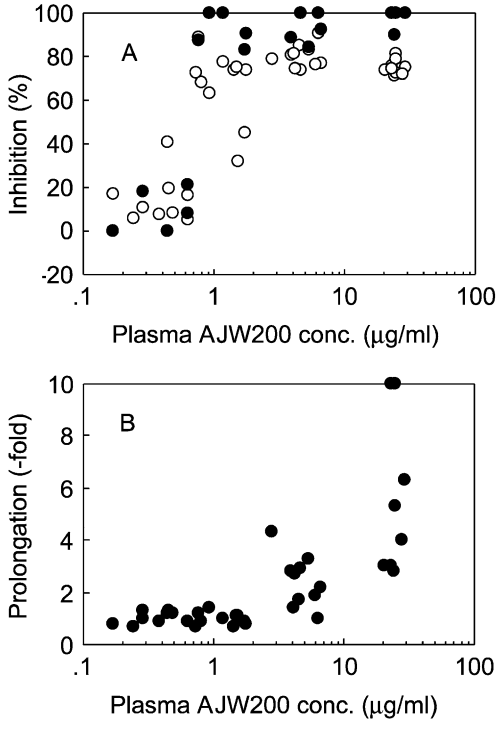

Fig. 5. (A) Correlation of plasma AJW200 concentration with the inhibition of ex vivo botrocetin-induced platelet aggregation (○) and inhibition of cyclic flow reductions (●). (B) Correlation of plasma AJW200 concentration with bleeding time prolongation.

4. Discussion

The present study demonstrates that AJW200, a humanized anti-vWF monoclonal antibody, inhibits thrombus formation in the stenosed coronary arteries without the prolongation of bleeding time, showing a safer profile than abciximab in dogs. Although GPIIb/IIIa antagonists including abciximab have been widely used for the treatment of patients with acute coronary syndromes, the clinically available dose is known to prolong the bleeding time extensively (Tcheng et al., 1994). Whereas the high shear stress-induced platelet aggregation was augmented via the increase in plasma vWF level in patients with acute coronary syndromes (Goto et al., 1999; Eto et al., 1999), suggesting that vWF-mediated platelet aggregation might be associated with the pathophysiological process of acute coronary syndromes. The GPIb–vWF blockers, including aurintricarboxylic acid, a recombinant vWF peptide fragment and murine anti-vWF monoclonal antibody (AJvW-2), inhibited the coronary arterial thrombosis in some experimental animal models (Strony et al., 1990; McGhie et al., 1994; Kageyama et al., 2001). However, no such agents have been available for clinical practices yet.

AJW200 is a humanized monoclonal antibody to vWF, and specifically inhibits vWF-mediated platelet reactions at the comparable concentrations to its parent antibody AJvW-2 (Kageyama et al., 2002). The present study confirmed that AJW200 had almost the same characteristic in vivo profile in a canine model of coronary arterial thrombosis as the parent monoclonal antibody, although the therapeutic window of AJW200 seemed to be a little narrow compared to that of AJvW-2 Fab. In the previous study, AJvW-2 Fab significantly inhibited cyclic flow reductions at 0.06 mg/kg (77% inhibition), although a significant prolongation of bleeding time was observed at 1.8 mg/kg (Kageyama et al., 2001). Whereas, AJW200 showed a significant inhibition of cyclic flow reductions at 0.1 mg/kg (92% inhibition) and a significant prolongation of bleeding time at 0.3–1 mg/kg in the present study. Because no significant effect (11% inhibition) was observed at 0.03 mg/kg AJW200, it was suggested that the efficacy of AJW200 on the coronary arterial thrombosis was almost comparable to that of AJvW-2 Fab in dogs. Therefore, the discrepancies between both studies would result from the difference of methodology of the bleeding time. Although the same template method using Simplate R was performed in both studies, the incision site was different. In the present study, the incision was performed at the exposed inner lip (mucosa bleeding). However, in the previous study using AJvW-2 Fab, the incision was performed at the surface of hindleg (skin bleeding). The region of incision site need to be washed, shaved and dried before the measurement. Also, dog's skin is too thick to incise reproducibly. To our experience, the bleeding time measured at the mucosa was likely to be prolonged, compared to that at the skin. Actually, the bleeding time was prolonged from 3.9 ± 0.4 s to 22.1 ± 3.6 s (≥ 30 min in two of five dogs) at the mucosa 5 min after administration of 0.8 mg/kg abciximab, although the same dose showed a less prolongation (from 3.8 ± 0.6 s to 9.0 ± 0.8 s) when measured at the skin (Kageyama et al., 2001). Despite of such differences, the present study reconfirmed that the blockade of the GPIb–vWF interaction potentially possesses a safer profile than the GPIIb/IIIa blockade.

These in vivo profiles of AJW200 may be in part explained by the difference of molecular mechanism between arterial thrombosis and hemostasis. Especially in the stenosed coronary artery, where the high shear stress is generated, platelet GPIb, GPIIb/IIIa and vWF, but not fibrinogen, are involved in platelet-thrombus formation (Ikeda et al., 1991). On the contrary, the hemostatic plug is likely to form under the low shear stress condition, where platelet GPIIb/IIIa and fibrinogen, as well as the coagulation cascade, may be involved. As a consequence of the high shear stress-dependent antithrombotic profile (Kageyama et al., 2002), AJW200 effectively prevents arterial thrombosis with a less prolongation of bleeding time. GPIIb/IIIa antagonists, however, work at both high and low shear and their bleeding problems may be due to the blockade of hemostasis at the low shear sites. Although this study has the limitation that the bleeding time has not been proven to be predictive of a risk for clinical bleeding episodes (Bernardi et al., 1993), such a safe profile of AJW200 will be preferable in clinical practices.

The antithrombotic effect of AJW200 well corresponded to the ex vivo antiplatelet effect. Approximately 70% inhibition of botrocetin-induced platelet aggregation results in the complete inhibition of cyclic flow reductions in this study. Whereas abciximab showed a complete inhibition of cyclic flow reductions at 79% inhibition of ADP-induced platelet aggregation, but not at 62% inhibition, which were almost accordant with the previous reports (Coller et al., 1989). Currently, the GPIIb/IIIa receptor occupancy has been often measured in patients treated with abciximab (Mascelli et al., 1997; Hézard et al., 1999). In patients with acute coronary syndromes (Tcheng et al., 1994) and animal models of coronary thrombosis (Coller et al., 1989; Gold et al., 1988), the ex vivo platelet aggregation was virtually abolished when GPIIb/IIIa receptor occupancy was maintained $\geq 80\%$. It is advantageous to monitor antiplatelet therapy to ensure that a therapeutic effect has been achieved initially and is sustained throughout the course of treatment. Also, after discontinuation of therapy, it may be important to know if platelet function has been restored. For the same purposes, we also measured not only the plasma concentration of AJW200, but also the vWF occupancy in this study, and investigated their correlation with the efficacy of AJW200 and the bleeding time prolongation.

Both the inhibition of cyclic flow reductions and of botrocetin-induced platelet aggregation, were observed at approximately 50% occupancy of vWF, and the bleeding time was extensively prolonged when 80–100% of vWF was occupied. Even though vWF occupancy was saturated, the bleeding times were not affected in some animals. On the contrary,

both the inhibition of cyclic flow reductions and of platelet aggregation were observed at approximately 0.7 μg/ml in plasma, and the bleeding time was extensively prolonged when plasma concentration reached approximately 20 μg/ml. These results suggest that vWF occupancy, as well as the plasma concentration, will be helpful parameters for additional AJW200 administration, for neutralizing AJW200 activity acutely, or in monitoring long-term AJW200 treatment.

In conclusion, the blockade of GPIb–vWF interaction with AJW200 results in a safer antithrombotic profile than the GPIIb/IIIa blockade with abciximab in dogs. The antithrombotic and antihemostatic effects of AJW200 were observed at 50% occupancy of vWF (0.7 μg/ml in plasma) and 80–100% occupancy (20 μg/ml in plasma) in dogs, respectively. AJW200 may become a promising drug for the treatment of patients with acute coronary syndromes.

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