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Anti-platelet and anti-thrombosis characteristics of Z4A5, a novel selective platelet glycoprotein IIb/IIIa inhibitor, compared with eptifibatide under long-term infusion

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Platelet Glycoprotein IIb/IIIa inhibitors are approved for the treatment of acute coronary syndromes and percutaneous coronary interventions due to their effects on the final common pathway of platelet aggregation. Z4A5 is a new hexapeptide IIb/IIIa inhibitor with antiplatelet and antithrombotic effects. This study was performed to assess the characteristics of Z4A5 compared with another IIb/IIIa inhibitor eptifibatide. Light-transmission aggregometry was used to measure platelet aggregation to assess the antiplatelet efficacy of Z4A5 *in vitro* and *ex vivo* in beagles. The time course of platelet inhibition and bleeding time prolongation during i.v. bolus plus infusion and after infusion of the Z4A5 were evaluated in beagles following two 2×2 Latin square designs. We also compared the antithrombotic activity of Z4A5 with eptifibatide in arterial thrombosis and arteriovenous shunt thrombosis model in beagles. Our data showed that Z4A5 completely inhibited adenosine diphosphate (ADP)-, thrombin- and arachidonic acid-induced *in vitro* platelet aggregation with values of IC_{50} of 260 nM, 128.6 and 56.4 n respectively. Z4A5 also markedly and stably prevented ADP-induced *ex vivo* platelet aggregation and prolonged the bleeding time throughout the 8-hour infusion. Both platelet function and bleeding time returned to normal sooner after cessation of Z4A5 infusion than after eptifibatide. Z4A5 inhibited thrombosis and had the same potent antithrombotic activity as eptifibatide. In conclusion, Z4A5 has the same potent antiplatelet effect and antithrombotic activity with the advantage of a faster on and off time compared to eptifibatide.

1. Introduction

Acute coronary syndromes (ACS) are often initiated by platelet activation. Glycoprotein (GP) IIb/IIIa inhibitors play an important role in the management of ACS by inhibiting the final common pathway of platelet aggregation (Shah et al. 2010). Three GP IIb/IIIa inhibitors are available in the global market: the specific antibody abciximab, the non-peptide tirofiban, and the cyclic heptapeptide eptifibatide (Kristensen et al. 2013). Several large randomised controlled trials have shown that eptifibatide significantly reduces the incidence of death or myocardial infarction. Eptifibatide may be particularly useful in the management of patients undergoing percutaneous coronary intervention (PCI) Pursuit trial investigators 1998; ESPRIT. 2000). The American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommend GP IIb/IIIa inhibitors as an integral component of care in these patients (Shah et al. 2010; Braunwald et al. 2002). Although GP IIb/IIIa inhibitors are the most potent anti-platelet agents adopted as adjuvant regimens, they have some potential disadvantages, such as slow recovery of platelet function, haemorrhage, and thrombocytopenia (Wang et al. 2011). Unforeseen findings of increased bleeding risk and recurrent arterial thrombosis have hampered the development of superior next-generation antiplatelet therapies. Attempts to develop new drugs in this class to overcome these disadvantages are ongoing.

Z4A5, a new platelet GP IIb/IIIa antagonist, is a linear hexapeptide with double arginine-glycine-aspartate (RGD) recognition sequences (Li et al. 2011). In our previous study, we found that Z4A5 significantly inhibits the platelet aggregation in blood from human and several other animals *in vitro* (Li et al. 2011; Jing et al. 2013; Jing et al. 2011). We also demonstrated that Z4A5 inhibits coronary and femoral artery thrombosis in canines after one hour of infusion. In addition, Z4A5 potentiates these antithrombotic effects when used in combination with aspirin (Jing et al. 2011, 2013). These observations suggest that Z4A5 could be used as an alternative treatment for coronary artery ischemic syndromes.

GP IIb/IIIa inhibitors are infused intravenously, usually for 48 h -72 h (Shah et al. 2010; Banno et al. 1999). We thought it necessary to observe the long-term effects of Z4A5 infusion under the GP IIb/IIIa inhibitor dose regimens usually given to patients. Besides the functional receptor dynamics of Z4A5, we also evaluated the anti-platelet properties of Z4A5 in beagles with long-term intravenous infusion compared with eptifibatide. Our findings indicate that Z4A5 inhibits ADP-, TH- and AA-induced

Table 1: Concentration of half-maximal (50%) inhibition of Z4A5 on in vitro platelet aggregation in blood from beagles (n = 8)

Group	IC_{50} values (nM)		
	ADP-induced	AA-induced	TH-induced
Z4A5	260	56.4	128.6
Eptifibatide	82	171.6	153.2

IC50: concentration of.50% maximal inhibition. ADP: adenosine diphosphate; TH: thrombin; AA: arachidonic acid.

platelet aggregation as potent as eptifibatide and that the platelet function suppressed by long-term infusion of Z4A5 recovers faster than eptifibatide.

2. Investigations and results

2.1. *Inhibitory efficacy and potency of Z4A5 on* in vitro *platelet aggregation in beagles*

The anti-platelet activities of Z4A5 were assessed in platelet aggregation induced by three different inducers, adenosine diphosphate (ADP), thrombin (TH), and arachidonic acid (AA) in beagles. In blood samples from beagles, Z4A5 displayed a dose-dependent inhibitory activity toward platelet aggregation. The IC_{50} values are shown in Table 1. The efficacy of eptifibatide against ADP-induced *in vitro* platelet aggregation was slightly stronger than that of Z4A5 while Z4A5 showed a stronger inhibitory activity in AA-induced *in vitro* platelet aggregation than that of eptifibatide. Z4A5 and eptifibatide showed the same potent activity in TH-induced *in vitro* platelet aggregation. The potency of the two drugs reached 100% inhibition, which means that these two drugs completely inhibited ADP-, TH- and AA-induced platelet aggregation. ADP was chosen to be the inducer for further *ex vivo* studies.

2.2. *Inhibitory effects of Z4A5 on ADP-induced platelet aggregation* ex vivo *in beagles*

There were no significant differences in platelet aggregation pre-dose between the two groups (Z4A5: 65.80 ± 2.33%; eptifibatide: 71.66 ± 6.70%, P > 0.05). The platelet aggregation decreased sharply after the bolus dose and remained at this level for 8 h during Z4A5 infusion (data not shown). Figure 1 summarises inhibition of platelet aggregation (IPA) in different groups at different time points during GP IIb/IIIa inhibitor infusion. Similar degrees of platelet suppression were achieved by eptifibatide. No significant difference in antiplatelet activity was observed between two groups at different infusion time points (P > 0.05). Z4A5 exhibited a fast onset and a similar potent and stable inhibition of platelet aggregation as eptifibatide at the dose of 100/5 (Fig. 1).

2.3. *Recovery of platelet function after Z4A5 cessation*

Figure 1 also summarises the results of the IPA at different time points after the termination of drug infusion. Recovery did not begin before discontinuation of the infusion. Gradual recovery of platelet function then occurred over time after the termination of drug infusion. Compared with eptifibatide, platelet function recovered tasks after termination of Z4A5 infusion (IPA 71.37% vs.96.86% at 5 min, 57.98% vs. 94.30% at 15 min, 26.05% vs. 83.79% at 30 min, and 9.75% vs. 56.86% at 60 min; P < 0.05, n = 4). The inhibition of platelet aggregation by eptifibatide decreased to 50% at 90 min after the termination of infusion,

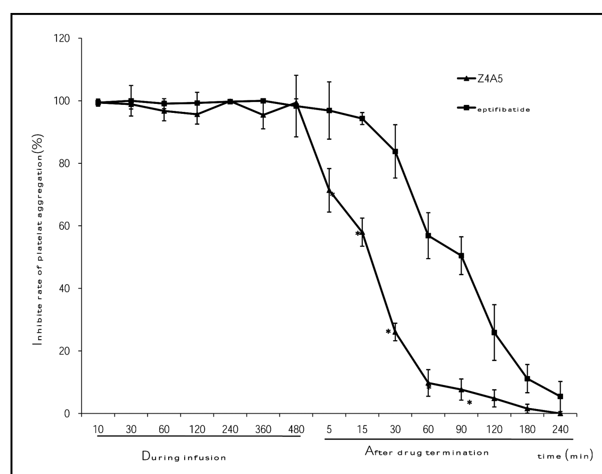

Fig. 1: Z4A5 inhibited platelet aggregation during 8 h of infusion. At dose of a 0.1 mg.kg^{-1} bolus followed by a 0.005 mg.kg^{-1} min^{-1} infusion for 8 h (100/5), Z4A5 (closed triangle) and eptifibatide (100/5, closed square) significantly inhibited ADP-induced canine platelet aggregation. Data are the mean ± Sd. There was no difference between Z4A5 and eptifibatide during 8-hour infusion. (P > 0.05, n = 4). The inhibition of platelet aggregation decreased gradually after the termination of drug infusion. Z4A5 (closed triangle) showed a significantly greater recovery of platelet function at 5, 15, 30,60 and 60 min after termination of infusion compared with eptifibatide (closed square) ($^{*}P < 0.05$,n = 4). Data are the mean ± Sd.

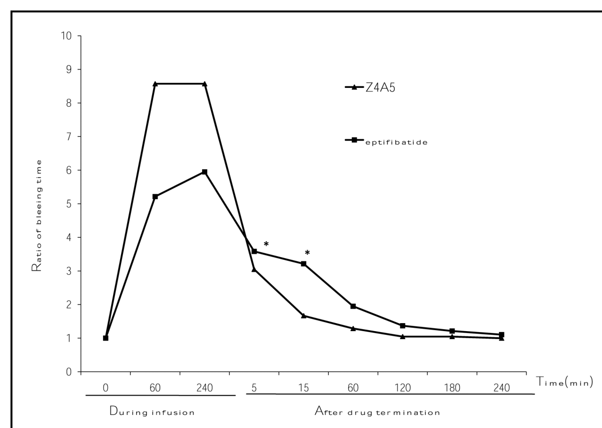

Fig. 2: Ratio of template bleeding time (TBT) extension of Z4A5 at different time points during drug infusion in beagles in a Latin square design. At the dose of a 0.1 mg.kg^{-1} bolus followed by a 0.005 mg.kg^{-1} min^{-1} infusion for 8 h (100/5), Z4A5 (closed triangle) and eptifibatide (closed square) significantly prolonged the template bleeding time. The bleeding time declined gradually after the termination of drug infusion. The ratio of bleeding time extension of Z4A5 at different time points during drug infusion was similar to that of eptifibatide. Ratio of bleeding time extension = post-dose bleeding time / pre-dose bleeding time. Compared with eptifibatide, Z4A5 showed a significantly greater recovery of bleeding time after termination of drug infusion at 5 and 15 min ($^{*}P < 0.05$, n = 4).

while inhibition was decreased to the same level by Z4A5 in only 15 min.

2.4. *Effect of Z4A5 on template bleeding time*

Figure 2 shows the results of the bleeding times in different groups at different time points. There were no significant differences in bleeding time pre-dose between the two groups (Z4A5: 2.3 ± 0.3 min; eptifibatide: 2.1 ± 0.8 min; P > 0.05, n = 4). During the 8 h of infusion at the rate of 100/5, the bleeding time was prolonged to 20 min in the Z4A5 group. The ratio of the bleeding time extension due to Z4A5 at different time points during drug infusion compared with baseline ranged from 5.6-6.6 (Fig. 2).

Table 2: Z4A5 inhibited thrombosis in arterial thrombosis model and arteriovenou thrombosis model ($x \pm s$, n = 4)

Group	Dose μg/kg(min)	Weight of AVST(mg)	Weight of AT(mg)
control	NS	312.0 ± 42.2	21.4 ± 7.1
Z4A5	100 + 5	174.9 ± 30.0*	9.5 ± 3.3*
Eptifibatide	100 + 5	166.7 ± 38.8*	8.9 ± 2.1*

Compare with control, *$P < 0.05$; compare with eptifibatide, $P = 0.896$.

Eptifibatide showed a similar effect on bleeding time, with a ratio that ranged from 5.0-5.9 with respect to baseline.

2.5. Recovery of template bleeding time after Z4A5 cessation

As shown in Fig. 2, the bleeding time declined gradually after the termination of drug infusion. Bleeding times were still 3.6 -, 4.3- and 1.9-fold prolonged at 5, 15, and 60 min after the termination of eptifibatide compared with the baseline, while the bleeding time declined to baseline quickly once Z4A5 infusion had been discontinued (Fig. 2).

2.6. Antithrombotic effects of Z4A5 on thrombus weight in beagle arterial model of thrombosis

The effect of antithrombotic agents on thrombus weight was assessed in a beagle femoral arterial model of thrombosis by extracting and weighing the thrombus 50 min after the application of $FeCl_3$. At the concentration tested, Z4A5 and eptifibatide effectively reduced weight of the thrombus. When compared to the control group, animals in the Z4A5 and eptifibatide -treated groups exhibited a significant reduction ($P < 0.05$) in thrombus weight (Table 2). Z4A5 has almost the same potent anti antithrombotic effects with an inhibitory rate of 55.8% compared to eptifibatide, 58.7%.

2.7. Antithrombotic effects of Z4A5 on thrombus weight in beagle arteriovenous shunt models of thrombosis

The effect of antithrombotic agents on thrombus weight was assessed in a beagle femoral arteriovenous shunt model of thrombosis by extracting and weighing the thrombus 40 min after opening of the blood vessel. At the concentration tested, Z4A5 and eptifibatide effectively reduced weight of the thrombus. When compared to the control group, animals in the Z4A5-and eptifibatide treated groups exhibited a significant reduction ($P < 0.05$) in thrombus weight (Table 2). Z4A5 has almost the same potent anti antithrombotic effects with an inhibitory rate of 44.0% compared to eptifibatide, 46.6%.

3. Discussion

This study shows that Z4A5 completely inhibited ADP-, TH- and AA-induced platelet aggregation *in vitro* with the same potency as eptifibatide in beagles. It also shows that Z4A5 markedly and stably inhibited *ex vivo* platelet aggregation and prolonged the bleeding time at a dose of 100 μgkg^{-1} (bolus) followed by a 5 $\mu gkg^{-1}min^{-1}$ infusion for 8 h. More importantly, the recovery of platelet function suppressed by long-term infusion of Z4A5 was faster after the termination of drug infusion than with eptifibatide. This result suggests that Z4A5 can be even better controlled than the other GP IIb/IIIa inhibitor.

The Latin square design conserves research subjects, animal or human, and yields rich information as well. Additionally, the significant difference in the observational index between drugs is more objective and reliable because the data are obtained from the same subject (Hu and Bao 2012). In this study we investigated the anti-platelet effects of eptifibatide and our newly designed GP IIb/IIIa inhibitor, Z4A5. Ten days of wash-out were sufficient for platelet recovery, both functionally and numerically, in beagles and for the elimination of these drugs.

A > 80% level of GP IIb/IIIa blockade with suppression of platelet aggregation to < 20% of baseline (>80% IPA) is sufficient to suppress platelet activity and, further, to prevent thrombus formation (Tcheng et al. 1994; Gold et al. 1988). In our study, the mean IPA reached 99.6% and remained at this level throughout the 8-hour infusion. Thus, Z4A5 shows an effective and stable inhibition of platelet aggregation.

Furthermore, platelet function began to recover within 5 min of the cessation of Z4A5 infusion; regardless of infusion duration (1 h (Jing et al. 2013) or 8 h (this study)). The results of TBT are consistent with the platelet function recovery data. Therefore, Z4A5 has a rapid onset and offset of platelet function inhibition. We think the rapid onset and offset are the most important characteristics of Z4A5 compared with other antiplatelet agents. The quickly reversible effect of Z4A5 minimises the likelihood of haemorrhagic problems during the post-treatment period which is essential for long-term safety. After the end of infusion of eptifibatide, restoration of normal platelet aggregation takes approximately 4 h (Schror and Weber 2003). Abciximab binds much more avidly to GP IIb/IIIa than these agents and has a measurable antiplatelet activity for several days (72 h) (Shah et al. 2010).Thus, it might be convenient for physicians to monitor antiplatelet therapy to administer the optimal dose for the prevention or treatment of thrombosis while minimising haemorrhagic side effects and to adapt the therapy and therapeutic schedule according to the patient's situation (Michelson and Frelinger 2006; Price 2009).

The results of this study are consistent with our pharmacokinetics studies which showed that Z4A5 has a shorter half-life ($t_{1/2}$) of 7 min - 12 min (submitted for publication) compared with eptifibatide (2.5 h).

$FeCl_3$ induced thrombosis model thus simulate intravascular thrombotic events and seems to be more useful in evaluating the anti-thrombotic drugs as well as in exploring the molecular mechanisms involved in arterial thrombosis (Couture et al. 2011). It is also a very simple model to use and does not require specialized surgical techniques or equipments to induce endothelium injury. Thrombus weight is one of the primary endpoints for assessing the efficacy of antithrombotic drugs. Consistent with results of platelet inhibition, Z4A5 inhibited thrombosis in two models which have relationship to platelet.

In conclusion, Z4A5 has a fast, marked, and sustained inhibitory effect on platelet aggregation in canines. The recovery of platelet function suppressed by Z4A5 was significantly more rapid than that suppressed by eptifibatide. Based on these data and our previous studies, Z4A5 is a potent GP IIb/IIIa inhibitor with advantageous properties, such as direct action, reversible binding, competitive binding, fast effects, constant effects over a long treatment course, quick recovery from its antiplatelet effects, and short half-life (submitted for publication). Given the lack of an effect on haemodynamic and coagulation parameters and the marginal increase in bleeding time, it is worth to develop this new antithrombotic compound for clinical use.

Table 3: The 2 × 2 Latin square design

Beagle dog number	Order	
	First turn	Second turn
1	A	B
2	B	A
3	A	B
4	B	A

The two letters represent two levels of the experiment factor type of drug. A represents Z4A5 which was infused as a bolus of 0.1 mg.kg-1 followed by progressively longer infusions at a rate of 0.005 mg.kg-1min-1 for 8 h (Z 100/5).B represents eptifibatide which was infused as a 0.1 mg.kg-1 bolus followed by a 0.005 mg.kg-1min-1 infusion for 8 h (E 100/5).

4. Experimental

4.1. Preparation of animals

Twelve healthy adult male Beagle dogs (9.0 kg -12.0 kg) were purchased from Xi'an Dilepu Biological Resources Development Co., Ltd. (Animal certificate number SCXKshaanxi 2012-001) and moved into the Experimental Animal Centre of Xi'an Jiaotong University, China. Dogs were housed individually in stainless-steel cages in a temperature- (21 °C) and light-controlled (14-h light: 10-h dark) room. Water and food were provided *ad libitum*. All of the experimental procedures were approved by the Xi'an Jiaotong University Committee for Animal Research and were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

4.2. Measurement of platelet function

Blood samples were prepared as described previously (Jing et al. 2013). Briefly, blood was collected from human or canine femoral arteries into a plastic syringe containing 3.8% sodium citrate to prevent coagulation. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation for 8 min at 100 *g* or for 10 min at 1860 *g*, respectively. Light-transmission aggregometry was used to measure platelet aggregation in a four-channel platelet aggregation analyser (LBY-NJ4, Beijing Precil Instrument Co., Ltd., China) by recording the increase in light transmission through a stirred suspension of PRP. ADP (20 μM), or TH (12 KU L^{-1}), or AA (20 μM) and adrenaline (Ad, 1 M) were used as platelet agonists. The results are expressed as the inhibition of platelet aggregation (IPA), calculated as: IPA% = 100 % × (pre-dose platelet aggregation–post-dose platelet aggregation)/pre-dose platelet aggregation.

4.3. Measurement of the potency and efficacy of Z4A5 against platelet aggregation in vitro

Platelet aggregation assays were performed using individual platelets from six different Beagle dogs. PPP and PRP were prepared as described above. Different concentrations of Z4A5 (3×10^{-9}, 1×10^{-8}, 3×10^{-8}, 1×10^{-7}, 3×10^{-7}, 1×10^{-6}, and 3×10^{-6} M) were assayed to calculate the concentrations resulting in 50% inhibition (IC_{50}) and the maximal effect (E_{max}) to assess the anti-platelet potency and efficacy of Z4A5 *in vitro*, respectively. IPA was calculated as above. E_{max} and IC_{50} values were obtained from dose-response curves for each dog and calculated as the mean IC_{50}.

4.4. Experimental design

Two 2 × 2 Latin square design was used for the *in vivo* experiment (Hu and Bao 2012). We choose four Beagle dogs from the same brood as the research subjects. Each dog received the two GP IIb/IIIa inhibitors in different orders at different times. Table 3 lists the design format.
The dose of eptifibatide for Beagles was calculated based on human clinical doses. There was a wash-out time of 10 days between each pair of treatments. The observational indexes were platelet aggregation and bleeding time at different time points during drug infusion and after the termination of drug infusion.

4.5. Animal surgery protocol

Beagles were anaesthetised with sodium pentobarbital (30 mg kg^{-1}) and maintained combine with xylazine (5 mg kg^{-1}). Body temperature was maintained at 37 °C with a heating table. A catheter was placed in either side of the upper-extremity vein for drug administration. Femoral arteries were cannulated for monitoring of mean arterial pressure and blood withdrawal. The dogs were allowed a 30-minute equilibration period after the surgery. Hydrogen peroxide and iodine were applied on the tongue and the incision occasionally for three days. Penicillin was given by intramuscular injection three times after the experiment to avoid infection.

4.6. Platelet aggregation at different time points

Fresh blood was collected to assess the platelet aggregation at different time points. Blood samples (3 ml) were obtained pre-dose (0 min); at 10, 30, 60, 120, 240, 360,and 480 min during drug infusion; and at 5, 15, 30, 60, 90, 120, 180, and 240 min after the termination of infusion. IPA was calculated as above.

4.7. Measurement of template bleeding time

The template bleeding time assays were performed as previously described (Jing et al. 2013)**.** An automated incision device (KJ119, Jiangsu Kangjian Medical Apparatus Co., Ltd.,China) was used to make a standardised incision 5 mm long and 1 mm deep on the upper surface of the tongue. The lesion was carefully blotted with filter paper every 20 s until bleeding ceased. Bleeding time was defined as the time elapsed until bleeding stopped. When bleeding continued beyond 20 min, the assay was terminated and the measurement was reported as a value truncated at 20 min. All bleeding assays were performed by the same operator at different times: pre-dose (0 min); at 60 and 240 min during drug infusion; and at 5, 15, 60, 120, 180, and 240 min after the termination of infusion. The ratio of bleeding time extension was calculated by the following equation: ratio of bleeding time extension = post-dose bleeding time / pre-dose bleeding time.

4.8. $FeCl_3$ induced arterial thrombosis model

This model was performed as described previously (Jing et al. 2013). Briefly, Beagles were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). A 3 cm - 4 cm segment of a left femoral artery was exposed and an electromagnetic flow probe (RM-6000, Nihon Kohden Corporation, Tokyo, Japan) was placed under the distal artery. A filter paper (1cm × 1.5 cm) saturated with 50% $FeCl_3$ solution was placed on the surface of the artery. Fifty minutes after the initial application of $FeCl_3$ the femoral artery was dissected and the thrombus was scraped out and measured immediately to record the wet weight. Drug or saline was given intravenously 1 min prior to application of ferric chloride.

4.9. Arteriovenous shunt thrombosis model

Along with the arterial model, an 8-cm polyethylene tube was inserted between the right femoral artery and the left femoral vein. The saline-filled shunt was assembled by connecting two cannulae with a slightly curved 12-cm-long tygon tubing (internal diameter 2 mm) containing a 8-cm-long cotton thread (4 operation silk thread) (Christopher et al. 1994). The extracorporeal circulation was maintained for 40 min, during which time a thrombus adheres to the cotton thread. The shunt was then removed and the thread with its associated thrombus was withdrawn and immediately weighed.

4.10. Drugs, chemical reagents, and other materials

The chemical structure of Z4A5 was reported previously Z4A5 was synthesised by solid-phase peptide synthesis methods with standard-functionality-protected amino acids as described previously (Jing et al. 2013). Compounds were purified to 99.8% purity by high-performance liquid chromatography and characterised by nuclear magnetic resonance, mass spectrometry, and aminoacid analysis. Z4A5 was dissolved in normal saline for injection. Eptifibatide was obtained from Shengnuo Biological Product Co., Ltd. (Chengdu, China). ADP, TH, AA and pentobarbital sodium were purchased from Sigma (St. Louis, MO, USA). Other reagents were domestic products and analytically pure.

4.11. Data analysis and statistical procedures

Scott's ratio method was used for linear regression to calculate the E_{max} and IC_{50} of Z4A5 against platelet aggregation *in vivo* (Sun 1987). For the platelet aggregation *ex vivo* study and the bleeding time *in vivo* study, we performed univariate analysis of variance for a Latin square design using SPSS 13.0 for Windows. A pair wise comparison was performed for each pair of groups. In all statistical tests, values of $P < 0.05$ were considered statistically significant.

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References

Banno H, Kawazura H, Yutaka T, Sakuma N, Kitamori T, Hosoya J, Kibayashi K, Yamashita H, Umemura K, Nakashima M (1999) Antiaggregatory, antithrombotic effects of ms-180, a novel platelet glycoprotein iib/iiia receptor antagonist. Eur J Pharmacol 367: 275–282.

Berry CN, Girard D, Lochot S, Lecoffre C (1994) Antithrombotic actions of argatroban in rat models of venous,mixed' and arterial thrombosis, and its effects on the tail transection bleeding time. Br J Pharmacol 113: 1209–1214.

Braunwald E, Antman EM, Beasley JW (2002) Acc/aha guideline update for the management of patients with unstable angina and non-st-segment elevation myocardial infarction–2002: Summary article: A report of the American college of cardiology/American heart association task force on practice guidelines (committee on the management of patients with unstable angina). Circulation 106: 1893–1900.

Couture L, Richer LP, Cadieux C, Thomson CM, Hossain SM (2011) An optimized method to assess in vivo efficacy of antithrombotic drugs using optical coherence tomography and a modified Doppler flow system. J Pharmacol Toxicol Meth 64: 264–268.

Gold HK, Coller BS, Yasuda T, Saito T, Fallon JT, Guerrero JL, Leinbach RC, Ziskind AA, Collen D (1988) Rapid and sustained coronary artery recanalization with combined bolus injection of recombinant tissue-type plasminogen activator and monoclonal antiplatelet gpiib/iiia antibody in a canine preparation. Circulation 77: 670–677.

Hu LP, Bao XL (2012) Three-factor designs unable to examine the interactions (part 2). J Chin Integr Med 10: 1229–1232.

Jing BB, Li YX, Zhang H, Ren ST, Wang M, Li YP, Shen XL, Wang YL, Zang WJ, Wang B (2013) Antithrombotic effect of Z4A5 on coronary thrombosis in a canine model of acute unstable angina. Br J Pharmacol 169: 848–859.

Jing BB1, Li YX, Zhang H, Ren ST, Wang M, Li YP, Zang WJ, Wang B (2011) Antithrombotic activity of Z4A5, a new platelet glycoprotein iib/iiia receptor antagonist evaluated in a rabbit arteriovenous shunt thrombosis model. Thromb Res 128: 463–469.

Kristensen SD, Würtz M, Grove EL, De Caterina R, Huber K, Moliterno DJ, Neumann FJ (2013) Contemporary use of glycoprotein iib/iiia inhibitors. Thromb Haemost 107: 215–224.

Li YX, Sun Q, Zhang H, Ren ST, Liao YR, Wang Y, Shen XL, Wang B (2011) A novel anti-platelet peptide (Z4A5) potential for glycoprotein iib/iiia inhibits platelet aggregation. Thromb Res 129: e217–222.

Michelson AD, Frelinger AL, 3rd, Furman MI (2006) Current options in platelet function testing. Am J Cardiol 98: 4N-10N.

ESPRIT investigators: Novel dosing regimen of eptifibatide in planned coronary stent implantation (esprit): A randomised, placebo-controlled trial (2000) Lancet 356: 2037–2044.

Price MJ (2009) Monitoring platelet function to reduce the risk of ischemic and bleeding complications. Am J Cardiol 103: 35A-39A.

Pursuit trial investigators: Inhibition of platelet glycoprotein iib/iiia with eptifibatide in patients with acute coronary syndromes(1998). Platelet glycoprotein iib/iiia in unstable angina: Receptor suppression using integrilin therapy. N Engl J Med 339: 436–443.

Schror K, Weber AA (2003) Comparative pharmacology of gp iib/iiia antagonists. J Thromb Thrombol 15: 71–80.

Shah I, Khan SO, Malhotra S, Fischell T (2010) Eptifibatide: The evidence for its role in the management of acute coronary syndromes. Core Evid 4: 9–65.

Sun RY. Quantitative pharmacology (1999) First ed. Peiping: People's Medical Publishing House, p.393–406.

Tcheng JE, Ellis SG, George BS, Kereiakes DJ, Kleiman NS, Talley JD, Wang AL, Weisman HF, Califf RM, Topol EJ (1994) Pharmacodynamics of chimeric glycoprotein iib/iiia integrin antiplatelet antibody fab 7e3 in high-risk coronary angioplasty. Circulation 90: 1757–1764.

Wang TY, White JA, Tricoci P, Giugliano RP, Zeymer U, Harrington RA, Montalescot G, James SK, Van de Werf F, Armstrong PW, Braunwald E, Califf RM, Newby LK (2011) Upstream clopidogrel use and the efficacy and safety of early eptifibatide treatment in patients with acute coronary syndrome: An analysis from the early glycoprotein iib/iiia inhibition in patients with non-st-segment elevation acute coronary syndrome (early acs) trial. Circulation 123: 722–730.