Clinical Investigation and Reports

Antiplatelet and Antithrombotic Efficacy of DMP 728, a Novel Platelet GPIIb/IIIa Receptor Antagonist

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Background Currently used antiplatelet drugs, including aspirin, ticlopidine, and others, are effective against certain but not all of the many endogenous platelet activators. Because of their limited efficacy, a significant number of serious thromboembolic complications still occur, highlighting the need for a more effective therapy. Thus, we have identified a systemically active peptide analogue (DMP 728) of the arginine-glycine-aspartic acid (RGD) recognition sequence that mediates the binding of ligands such as fibrinogen to the platelet glycoprotein (GP) IIb/IIIa receptors. The goals of the present study were to determine the antiplatelet and antithrombotic efficacies of DMP 728 in various arterial thrombosis models.

Methods and Results DMP 728 demonstrated antiplatelet efficacy in vitro in inhibiting ADP-induced human platelet aggregation (IC50, 46±2 nmol/L) and fibrinogen binding to human platelets (IC50, 2.3±0.8 nmol/L) or purified human GPIIb/IIIa receptors (IC50, 0.6±0.1 nmol/L). DMP 728 demonstrated high affinity and specificity for human platelet GPIIb/IIIa over other adhesion molecules. In anesthetized mongrel dogs, DMP 728 at 0.001 to 1.0 mg/kg IV produced dose-dependent antiplatelet effects in inhibiting ex vivo platelet aggregation induced by ADP and in prolonging template bleeding time. DMP 728 effects on bleeding time prolongation were more rapidly reversible than those on platelet aggregation inhibition. A maximal antiplatelet effect for DMP 728 was demonstrated at 0.01 mg/kg IV bolus. The antithrombotic efficacy of DMP 728 was examined in vitro and in vivo after IV administration at different doses in various models of arterial thrombosis. In the coronary artery Folts model in dogs, DMP 728 demonstrated maximal antithrombotic efficacy at 0.01 mg/kg IV bolus with an E50 of 0.005 mg/kg IV bolus in inhibiting cyclic flow reductions. Additionally, DMP 728 demonstrated 100% prevention of primary thrombosis and re-thrombosis (P<.01) after treatment with different thrombolytics, including tissue plasminogen activator and streptokinase, in an electrocytically induced femoral artery thrombosis model in dogs.

Conclusions Acute intravenous DMP 728 administration (0.001 to 1.0 mg/kg) has dose-dependent antiplatelet and antithrombotic effects in different arterial thrombosis models. These data suggest that DMP 728, a low-molecular-weight GPIIb/IIIa receptor antagonist, may have therapeutic potential as an effective antithrombotic agent in coronary and peripheral artery thromboembolic disorders. (Circulation. 1994;89:3-12.)

Key Words • antiplatelet agents • antithrombotic agents • glycoproteins • thrombosis • DMP 728

Platelet activation and the resulting aggregation have been shown to be associated with various pathophysiological processes, including cardiovascular and cerebrovascular thromboembolic disorders such as unstable angina, myocardial infarction, transient ischemic attack, stroke, and atherosclerosis. The contribution of platelets to these disease processes stems from their ability to form aggregates or platelet thrombi as a consequence of arterial wall injury. Injury of blood vessel walls could occur either acutely or chronically by various pathophysiological processes. Platelets are then activated by a number of activators or agonists that are released from within the platelets or from the injured arterial walls, with subsequent adherence, aggregation to the disrupted vessel surface, and resultant formation of an occlusive thrombus in the lumen of the vessel.

Current antiplatelet drugs are mainly effective against one of the many platelet activators. These drugs include aspirin, which blocks cyclooxygenases; ticlopidine, which acts against ADP; thromboxane A2 synthetase inhibitors or receptor antagonists, which act against thromboxane A2; and hirudin, which acts against thrombin. Hence, the potential clinical benefits of an agent that inhibits platelet activation in response to all of these agonists should represent a more efficacious therapeutic approach than the use of current platelet inhibitors, either alone or in combination. Additionally, a higher incidence of coronary artery reocclusion after successful thrombolytic therapy is a persistent clinical problem. Thus, prevention of reocclusion with an adjunctive pharmacological agent is an area that is being actively pursued with different compounds, including anticoagulants, antiplatelet agents, and maintained infusion of thrombolytics.

The platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa) has recently been identified as the final common pathway for all agonists. The binding of adhesive proteins, such as fibrinogen, to GPIIb/IIIa causes plate-
Platelet Aggregation Assay

Venous blood was obtained from anesthetized mongrel dogs or from healthy human donors who were drug- and aspirin-free for at least 2 weeks before blood collection. Blood was collected into citrated Vacutainer tubes. The blood was centrifuged for 15 minutes at 150g (850 rpm) in a Sorvall RT6000 tabletop centrifuge with H-1000 B rotor at room temperature, and platelet-rich plasma (PRP) was removed. The remaining blood was centrifuged for 15 minutes at 150g (26780 rpm) at room temperature, and platelet-poor plasma (PPP) was removed. Samples were assayed on a PAP-4 Platelet Aggregation Profile with PPP as the blank (100% transmittance). PRP (200 μL) (5 x 10^6 platelets/μL) was added to each micro test tube, and transmittance was set to 0%. ADP (20 μL) (10 μmol/L) was added to each tube, and the aggregation profiles were plotted (percent transmittance versus time). DMP 728 (20 μL) was added at different concentrations before the addition of the platelet agonist. Results are expressed as percent inhibition of agonist-induced platelet aggregation.

Platelet-Fibrinogen Binding Assay

Binding of 125I-fibrinogen to platelets was performed as described by Bennett and Vilaire, with some modifications as described below. Human PRP (h-PRP) was applied to a sepharose column to prepare human gel-purified platelets (h-GPP). Aliquots of h-GPP (5 x 10^6 platelets/μL) along with 1 mmol/L calcium chloride were added to removable 96-well plates. 125I-fibrinogen (26.5 μCi/μg) was added, and the h-GPP were activated by addition of the previously described platelet agonists. The 125I-fibrinogen bound to the activated platelets was separated from the free form by centrifugation and then counted on a gamma counter. Nonspecific binding (due to entrapment of 125I-fibrinogen) in either the presence or absence of the inhibitors was shown to be in the range of 4% to 6% of total 125I-fibrinogen binding to activated platelets. Percent inhibition of 125I-fibrinogen binding to activated platelets was calculated by dividing the specific binding (ie, total binding minus nonspecific binding) obtained in the presence of inhibitors by that obtained in the absence of the inhibitors. For IC50 evaluation, DMP 728 was added at various concentrations before platelet activation.

Metabolism/Stability Study

The potential metabolism of DMP 728 by microsomal and nonmicrosomal enzymes obtained from various species, including rats, dogs, and humans, was determined. The metabolic effect of standard proteolytic enzymes such as pepsin, trypsin, enteropeptidases, peptidases, and pseudoproteases was determined as well. DMP 728 was prepared as a high-performance liquid chromatography (HPLC) with on-line radiochemical detection. The metabolic viability of the different enzyme systems used was confirmed by incubating each enzyme with its known standard substrate under the same conditions as used for DMP 728. Additionally, the effects of different pHs, including 3.0, 7.4, and 8.5, on the stability of DMP 728 were examined by HPLC with UV detection. DMP 728 (100 μg/mL) was incubated in 50 mmol/L phosphate buffer at the above pHs for up to 6 hours. After incubation, the remaining DMP 728 was quantified by HPLC with UV (210 nm) detection.
Plasma Protein Binding Study

The in vitro binding of DMP 728 to human or canine plasma proteins was determined with an ultrafiltration technique and HPLC for the quantification of bound/free DMP 728. DMP 728 (10 to 100 µg/mL) was incubated for up to 4 hours at 37°C with plasma (DMP 728 or saline, control). Bound DMP 728 was separated from free DMP 728 by an ultrafiltration membrane filter with a 20,000 kD molecular weight cutoff. The free DMP 728 in the filtrate was quantified by HPLC with UV (210 nm) detection.

Antiplatelet Effects in Anesthetized Dogs

**Experimental Procedures.** Mongrel dogs of either sex weighing between 8 and 12 kg were anesthetized by intravenous administration of pentobarbital sodium solution (30 mg/kg) (Abbott Laboratories, 50 mg/mL vial). Animals were placed on a respiratory pump (model 665, Harvard Apparatus, South Natick, Mass) with the stroke volume adjusted to the dog's weight (stroke volume=dog's weight in kilograms times 15 mL/kg), and the respirator dials were set on 20 beats per minute and 50% respiration. Both femoral arteries were cannulated, one was attached to a transducer by which heart rate and blood pressure were monitored on a chart recorder (Gould Recorder 2800S, Gould, Cleveland, Ohio) throughout the study, and the other was used for repeated blood sampling. The femoral vein was also cannulated to administer DMP 728 or vehicle and for maintenance of anesthesia as needed.

**Experimental Protocol.** Animals were dosed intravenously (into the cannulated femoral vein over a period of 2 minutes after a basal blood sample) with DMP 728. DMP 728 was administered at different doses (0.001 to 1.0 mg/kg IV bolus). Bleeding time was determined before and at different intervals after DMP 728. Blood samples were collected into Vacutainer tubes (4.5 mL draw, each containing 0.5 mL of 0.05 mol/L sodium citrate) before and at different intervals after administration of DMP 728. Blood samples were placed on a platform rocker until the end of the study, and the samples were then assayed for platelet aggregation, platelet counts, and DMP 728 plasma levels. Plasma levels of DMP 728 were measured by an ELISA and by HPLC with a fluorescence detection method developed in our laboratory.

**Bleeding Time.** The effect of DMP 728 on bleeding time (minutes) was assessed on the back side of the tongue. A Simplate device (Organo Teknika) was applied to the surface of the tongue to make a uniform incision, and blood was blotted with filter paper at 30-second intervals until bleeding completely stopped, with a 15-minute cutoff time. Bleeding time was assessed before and at different intervals after administration of DMP 728.

**Platelet Counts.** From each blood sample, 20 µL of PRP was added to 20 mL of Isoton buffer (Coulter Diagnostics), and platelet number was determined with a Coulter Counter (Coulter Electronics, Inc.).

In Vitro Arterial Thrombosis Model

Thoracic aortas were dissected from anesthetized male New Zealand White rabbits and mounted in a tissue dish containing Tyrode's buffer (in mmol/L: NaCl 27.4, KCl 1.4, KH2PO4 0.64, MgSO4 1.2, H2O 1.2, CaCl2 0.6, NaHCO3 8.4, dextrose 7.2, pH 7.4) bubbled with 95% O2/5% CO2. The aorta was attached with sutures to tubing connectors projecting from the sides of the dish. Fig 2 is a sketch illustrating the design of the system used. Human whole blood collected in citrate tubes as previously described was preincubated at 37°C for 15 minutes with subthreshold (10−7 mol/L) epinephrine to facilitate the formation of a platelet-rich thrombus and then perfused through tubing connected to both ends of the vessel. Initial flow was set to 10 mL/min, and then the system was allowed to stabilize for 10 minutes. The arterial segment was injured either by the use of an internal (balloon-type angioplasty) or an external (mechanical clamping) means, along with the application of 60% to 70% constriction distal to the injured site. Arterial flow (mL/min) was monitored throughout the experiment by placing an electromagnetic flow probe connected to a flow meter (Carolina Medical Electronics, NC) around the distal portion of the vessel segment. This method resulted in repeated cycles of flow reduction (CFR) followed by restoration of flow upon dislodging or gentle shaking around the clamp. Basal measurements of the frequency of CFR (6 CFR/h) were recorded, either saline (control group) or test agent (treated groups) was then added at different concentrations to the blood tank, and the frequency of CFR was monitored throughout the study for up to 3 hours after addition of saline or test agent. The effects of DMP 728 or RGDS as well as other antiplatelet drugs such as aspirin or ticlopidine on the frequency of platelet-mediated CFR were evaluated.

Coronary Artery Thrombosis in Dog (Folts Model)

The in vivo coronary artery platelet-rich thrombus model has been described previously in detail.20−27 Twenty purpose-bred mongrel dogs of either sex weighing between 8 and 15 kg were anesthetized and handled as previously described. Additionally, a left thoracotomy was performed at the fifth intercostal space, and the heart was then placed in a pericardial cradle. The proximal left circumflex coronary artery (LCx) was dissected and freed from fascia and branches for a distance of 15 to 20 mm. A Doppler flow probe was placed around the distal portion of the vessel segment, and coronary flow was monitored throughout the study. Animals were allowed to stabilize for 20 minutes, and the hyperemic response of the dissected LCx was determined by two repeated brief (20-second) total occlusions 3 to 5 minutes apart. After restoration of basal flow for 20 minutes, a plastic cylinder 2.5 mm long was placed on the proximal portion of the LCx, creating a critical stenosis that reduced the lumen area of the vessel up to 80%, thereby preventing the hyperemic response while minimally affecting basal flow. The clip was then moved to one side, and a portion of the LCx was mechanically damaged by gentle clamping of the vessel. The stenotic clip was then moved back onto the damaged segment of the vessel. This resulted in repeated CFRe followed by restoration of flow upon dislodging or gentle shaking around the clip. Basal measurements of the frequency of CFR, heart rate, and mean arterial blood pressure were recorded, and then either saline (control group) or
DMP 728 (treated groups) was administered intravenously. The previously described parameters were monitored for up to 3 hours after administration of saline or DMP 728.

Femoral Artery Thrombosis in Dogs (Electrolytic Injury Model)

The model used in this study is a modification of the one described by Romson et al.26 The experimental procedure results in the formation of a platelet-rich intravascular thrombus at the site of the electrolytically induced lesion. The femoral artery response to the electrolytic injury is similar to that observed in the canine coronary artery, in which intimal wall injury secondary to application of a direct anodal current leads to platelet adherence, with resultant occlusive thrombus formation.28-29 Animals were instrumented as described earlier. A 20- to 30-mm segment of the femoral artery was exposed and freed from fascia, and branches were tied. Anodal current was applied with an intravascular electrode composed of a Teflon-insulated, silver-coated copper wire (28 gauge). Penetration of the vessel wall by the electrode was facilitated by attaching the tip of a 23-gauge hypodermic needle to the uninsulated part of the electrode. Each intra-arterial electrode was connected to the positive pole (anodal) of a dual channel stimulator (nickel-cadmium battery, 9 V, connected to a 250 000-Ω potentiometer in series). The cathode was connected to a distant subcutaneous site. The current delivered to the arterial wall was monitored continuously and maintained at 150 μA. Proper positioning of the electrode in the femoral artery was confirmed by visual inspection at the end of each experiment. In all experiments, the anodal current was applied for a maximum period of 3 hours. Arterial flow was monitored throughout the experiment by an electromagnetic flow probe connected to a flow meter (Carolina Medical Electronics) placed around the distal portion of the vessel segment.

Prevention of Rethrombosis After Thrombolysis

The anodal current was applied to the femoral artery for a maximum period of 3 hours or was terminated 15 minutes after blood flow in the vessel had remained stable at zero flow velocity (ie, stable occlusive thrombus formation). Then DMP 728 was administered at 1.0 mg/kg IV bolus at 30 minutes before tissue-type plasminogen activator (t-PA) or streptokinase (SK). The dose regimens of the thrombolytics were as follows: TPA was administered at 0.02 mg/kg intra-arterially (loading dose), followed by intra-arterial infusion of 0.003 mg·kg⁻¹·min⁻¹ in the canine coronary artery. SK was administered at 10 000 UI/kg IV (loading dose), followed by a constant infusion at 300 U·kg⁻¹·min⁻¹ until lysis occurred. Animals were followed for 4 hours after thrombolysis. At the end of the study, the vessel segment was ligated both proximal and distal to the point of injury and removed without disturbing the intravascular thrombus. The vessel segment was opened along its length, and the intact thrombus mass was lifted off the intimal surface of the vessel. The weight of the thrombus mass was determined from an analytical balance. The protocol for this series of experiments is summarized in Fig 3. Reperfusion after thrombolysis was defined as the restoration of flow to 80% of control values. Blood pressure, heart rate, and femoral artery blood flow were monitored throughout the experiment.

Statistical Analysis

Data are expressed as mean±SEM. The design of the experimental protocol in certain sections of the study allowed each animal to serve as its own control with regard to the basal values (percent aggregation and basal bleeding time). Data were analyzed by either paired or group analysis using Student's t test or ANOVA when applicable; differences were considered significant at P<.05.

Results

In Vitro Antiplatelet Effects

Platelet Aggregation. In vitro studies in PRP obtained either from mongrel dogs or human volunteers showed that DMP 728 inhibited platelet aggregation induced by 10 μmol/L ADP with IC₅₀ of 15 and 46 nmol/L, respectively (Table 1). In contrast, the linear peptide RGDS demonstrated antiplatelet effects with an IC₅₀ of 100 to 150 μmol/L. The responsiveness of the platelet GPIIb/IIIa antagonist varied among different species, with DMP 728 relatively ineffective in inhibiting platelet aggregation in PRP from rats (Table 1). A steep dose-response relation was demonstrated for DMP 728 in inhibiting platelet aggregation in either human or canine PRP (data not shown).

125I-Fibrinogen Platelet Binding. In the h-GPP 125I-fibrinogen binding assay, DMP 728 inhibited 125I-fibrinogen binding to activated h-GPP with an IC₅₀ of 2.3 nmol/L (Table 1). A slightly greater inhibitory efficacy for DMP 728 in inhibiting fibrinogen binding was shown in canine platelets compared with that in human platelets (Table 1). DMP 728 demonstrated similar potency

<table>
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<tr>
<th>Species</th>
<th>PRP Aggregation</th>
<th>125I-Fibrinogen Binding</th>
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</thead>
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<tr>
<td>Human</td>
<td>46±2</td>
<td>2.3±0.8</td>
</tr>
<tr>
<td>Mongrel dogs</td>
<td>15±3</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>Rats</td>
<td>&gt;10 000</td>
<td>&gt;10 000</td>
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</table>

Platelet aggregation (light transmittance aggregometry) was induced by adding the various agonists to platelet-rich plasma (PRP). 125I-Fibrinogen binding to human gel purified platelets stimulated with ADP, arachidonic acid, epinephrine (100 μmol/L each) and collagen (20 μg/mL). | Data represent mean IC₅₀ (nmol/L), n=3 to 5. |
(IC$_{50}$=2 to 5 nmol/L) in inhibiting $^{125}$I-fibrinogen binding to h-GPP regardless of the agonist used. Under the same assay conditions, the linear peptide RGDS or the natural ligand fibrinogen demonstrated a weak inhibitory efficacy with an IC$_{50}$ of 10 to 30 μmol/L. DMP 728 not only prevented $^{125}$I-fibrinogen binding but also displaced $^{125}$I-fibrinogen already bound to activated platelets (data not shown). This suggests a high affinity for DMP 728 in displacing fibrinogen binding in a platelet-rich clot.

Metabolism/Stability

No apparent metabolism of DMP 728 was found by either microsomal, nonmicrosomal, or standard proteolytic enzymes such as pepsin, trypsin, enterokinases, proteases, and peptidases. After incubation at the optimal pH, temperature, and buffer conditions for the different enzyme systems to be tested, the $[^3H]$DMP 728 remaining was quantified by HPLC with on-line radiochemical detection. The recovery of total DMP 728 radioactivity when incubating with or without the previously described enzymes ranged from 90% to 98%. The metabolic viability of the different enzyme systems used was confirmed by incubating each enzyme with its known standard substrate under the same conditions as used for DMP 728. Additionally, there was no apparent effect of the different pHs (3.0, 7.4, and 8.5) tested on the stability of DMP 728 as determined by HPLC with UV detection. The recovery ranged from 90% to 95% of total DMP 728 when incubated at the different pHs tested.

Plasma Protein Binding

DMP 728 (10 to 100 μg/mL) incubated with plasma proteins or saline (control) at 37°C for up to 4 hours showed minimal plasma protein binding. Bound DMP 728 was separated from free by an ultrafiltration membrane filter with a 20 000-kD molecular weight cutoff. The filtrate (free) was quantified by HPLC with UV (210 nm) detection. The percent DMP 728 bound to plasma proteins ranged from 15% to 25% at the different concentrations of DMP 728 tested.

In Vivo Antiplatelet Efficacy In Dogs

The antiplatelet efficacy of DMP 728 was determined in anesthetized mongrel dogs at doses ranging from 0.001 to 1.0 mg/kg IV bolus by monitoring ex vivo ADP-induced platelet aggregation (percent inhibition), bleeding time (minutes), platelet counts, and DMP 728 plasma levels. The duration of antiplatelet effect of DMP 728 was shown to be dose dependent. DMP 728 given to dogs at 0.01 mg/kg IV bolus exhibited maximal efficacy in inhibiting platelet aggregation. DMP 728 was shown to have minimal antiplatelet efficacy at 0.001 mg/kg IV bolus. DMP 728 (1.0 mg/kg IV bolus) demonstrated maximal and sustained antiplatelet effects (100% inhibition of platelet aggregation, >15 minutes prolongation of bleeding time) for up to 6 hours (Table 2). DMP 728 did not affect platelet count, red blood cell count, white cell count, or hematocrit when administered at 0.1 to 1.0 mg/kg IV bolus in anesthetized dogs (Table 3). Additionally, DMP 728 was without any effects on the different coagulation parameters, such as prothrombin time, thrombin time, or activated partial thromboplastin time (data not shown). A close correlation between the in vitro and ex vivo antiplatelet inhibitory efficacy of DMP 728 was noted based on the plasma levels (Tables 1 and 2). At plasma concentrations of 10 to 15 ng/mL, DMP 728 resulted in 50% to 60% inhibition of ex vivo platelet aggregation without any significant effects on bleeding time prolongation in anesthetized dogs. A maximal inhibition of ex vivo platelet aggregation along with 1.5- to 2.0-fold prolongation in bleeding time over basal values was shown at 35 to 45 ng/mL plasma levels of DMP 728 (Table 2).

In Vitro Antithrombotic Efficacy

Perfusion of human blood through a mechanically injured (external clamping) isolated rabbit aorta was shown to result in CFR. DMP 728 demonstrated total prevention of CFR at 0.1 μmol/L, with no effect on CFR at 0.01 μmol/L. The antithrombotic effects of other standard antiplatelet agents, including aspirin and ticlopidine as well as the GPIIb/IIIa prototype antagonist RGDS, were evaluated. RGDS at 1.0 μmol/L demonstrated 100% antithrombotic efficacy in reducing CFR, compared with maximal antithrombotic efficacy with DMP 728 at 0.1 μmol/L. In contrast, neither aspirin nor ticlopidine showed any distinct antithrombotic efficacy at concentrations up to 1.0 μmol/L (Fig 4). Additionally, in the same model but with a balloon-induced intimal wall injury, DMP 728 maximally inhibited both platelet deposition and arterial flow reduction at 0.1 to 1.0 μmol/L (data not shown). DMP 728 at 0.01 and 0.1 μmol/L corresponds to 10% to 20% and 100% inhibition of in vitro human platelet aggregation, respectively.

In Vivo Antithrombotic Efficacy in Dogs

Coronary Artery Thrombosis in Dog (Folts Model).

In this model of unstable angina, a platelet-dependent thrombus is produced in a mechanically injured coronary artery (LCx) in the presence of a high degree of arterial constriction.26,27 Under these conditions, coronary blood flow is occluded as a platelet-rich thrombus forms, which is followed by spontaneous dislodging of the thrombus in a cyclic manner (CFR). This study examined the antithrombotic efficacy of DMP 728 at 0.005 and 0.01 mg/kg IV bolus in this platelet-mediated thrombosis model. In 20 anesthetized dogs, arterial injury along with a high degree of stenosis of the LCx resulted in cyclic platelet-rich thrombus formation, leading to repeated CFR. In saline-treated animals (n=11), CFR of seven or eight per hour continued consistently throughout the experiment. DMP 728 at 0.005 mg/kg IV bolus (n=4) and 0.01 mg/kg IV bolus (n=5) prevented CFRs in 2 of 4 and in 5 of 5 dogs, respectively (Fig 5). DMP 728 demonstrated both maximal antiplatelet and antithrombotic efficacies at 0.01 mg/kg IV bolus in anesthetized dogs (Table 2 and Fig 5). The onset for the antithrombotic effects of DMP 728 was immediate, with a dose-dependent duration of action.
TABLE 2. Antiplatelet Effects of Intravenous DMP 728 in Anesthetized Mongrel Dogs

<table>
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<th>Dose Levels, mg/kg IV bolus</th>
<th>Time, min</th>
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<th>Bleeding Time, † min</th>
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Data represent mean±SEM, n=3 to 6 in each group.

*Ex vivo percent inhibition of platelet aggregation (PA) in platelet-rich plasma induced by ADP (10 μmol/L) over time using light transmittance aggregometry.

†Template bleeding time (minutes).

‡Plasma concentrations of DMP 728 as determined by an ELISA in the case of the 0.001 to 0.01 mg/kg IV bolus doses and by high-performance liquid chromatography in the case of the 0.1 and 1.0 mg/kg IV bolus doses.

reocclusion, time to reocclusion, time to reperfusion, and on thrombus weight after thrombolysis (SK or TPA) of an electrolytically induced thrombus in a canine femoral artery model were examined. DMP 728 administered at 1.0 mg/kg IV bolus before administration of SK or TPA resulted in total prevention of

TABLE 3. Effects of DMP 728 on Whole Blood Cell Counts in Anesthetized Dogs

<table>
<thead>
<tr>
<th>Dose/Time</th>
<th>Platelet Count, 10⁹/mm³</th>
<th>Red Cell Count, 10⁹/mm³</th>
<th>Hematocrit, %</th>
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<td>Baseline</td>
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<td>240 min</td>
<td>289±41</td>
<td>5.0±0.7</td>
<td>35.5±3.3</td>
</tr>
<tr>
<td>1.0 mg/kg IV bolus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>343±38</td>
<td>6.0±0.3</td>
<td>41.0±2.6</td>
</tr>
<tr>
<td>60 min</td>
<td>331±34</td>
<td>6.4±0.4</td>
<td>41.4±3.9</td>
</tr>
<tr>
<td>240 min</td>
<td>302±26</td>
<td>5.4±0.2</td>
<td>37.4±1.2</td>
</tr>
</tbody>
</table>

Normal cell count ranges in mongrel dogs: platelet count, 280 to 340; red cell count, 4.5 to 6.5; hematocrit, 26% to 42%. All values are mean±SEM, n=6 in each group.
reocclusion ($P < .01$), which occurs normally at 42±10 and 32±9 minutes after lysis, respectively (Fig 6 and Table 4). Interestingly, a steady and stable arterial flow in the DMP 728–treated group was noted, compared with an oscillatory flow with the occurrence of CFR in the saline/TPA- or the saline/SK-treated groups. The oscillations in flow were more frequent along with a faster reperfusion time (20 to 40 minutes) in the saline/TPA group compared with the saline/SK-treated group, in which reperfusion occurred at 60±20 minutes. Additionally, DMP 728 significantly ($P < .01$) shortened the time to reperfusion when given in conjunction with SK (11.4±3.3 minutes) compared with SK alone (60±20 minutes). DMP 728 resulted in a significant reduction ($P < .01$) in the weight of the thrombus formed when given in conjunction with either SK or TPA, compared with the saline-treated groups (Table 4). A significant prolongation of the time to reocclusion to >240 minutes (ie, 100% prevention of the incidence of reocclusion for up to the maximum period of the study) was demonstrated (Fig 6). The 1.0 mg/kg IV bolus dose of DMP 728 administered under this protocol is a supermaximal dose, based on the in vivo antiplatelet effects and the plasma levels as shown in Table 2. A lower dose level of DMP 728 might be enough for maximal antithrombotic efficacy in this model. These studies suggest the potential utility of DMP 728 as an adjunct to standard thrombolytics.

**Discussion**

It is well recognized that the platelet GPIIb/IIIa, via its binding to circulating fibrinogen, is the final common pathway for all agonist-induced platelet aggregation.6-7,17 The binding of fibrinogen is mediated in part by the RGD recognition sequence, which is common to other adhesive proteins that bind to GPIIb/IIIa receptors or other integrins.17 Several RGD-containing peptides, as well as the 7E3 monoclonal antibody against platelet GPIIb/IIIa, have been shown to block fibrinogen binding and prevent the formation of platelet thrombi.10-14 However, some problems are associated with the use of peptides as antithrombotic agents, including their short survival time or metabolic instability in the circulation, which might be disadvantageous in permitting rapid reversal of the antihemostatic effect in bleeding but clearly poses a burden in terms of dosing. This is not the case with DMP 728, which is metabolically stable and very effective at extremely low dose levels. In contrast to other antiplatelet agents such as aspirin, ticlopidine, and hirudin, which are effective mainly against a single agonist (arachidonic acid, ADP, or thrombin, respectively) in inhibiting platelet aggregation in human PRP,30 DMP 728 demonstrated high affinity and similar potency in inhibiting platelet aggregation regardless of the agonist used.31 Additionally, DMP 728 demonstrated a high degree of selectivity toward the platelet GPIIb/IIIa receptors compared with the closely related vitronectin receptors on endothelial cells or other adhesion receptors on platelets or leukocytes.31,32 DMP 728 is shown to be metabolically stable under various conditions. In vivo studies in rats and dogs demonstrated a lack of metabolism. Additionally, a minimal and perhaps insignificantly (15% to 25%) of plasma protein binding was demonstrated with DMP 728. Intravenous administration of DMP 728 in anesthetized dogs produced a dose-

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**Fig 4.** Antithrombotic effects of DMP 728 in an in vitro arterial thrombosis model. Data represent mean±SEM, n=3 or 4 in each group. Basal measurements of the frequency of cyclic flow reductions (CFR) were recorded, either saline (control group) or test agent (treated groups) was added at different concentrations to the blood tank, and then the frequency of CFR was monitored throughout the study for up to 3 hours. The effect of DMP 728 or RGDS as well as other antiplatelet drugs, including aspirin or ticlopidine, on the frequency of platelet-mediated CFR was evaluated in this model. Data represent mean±SEM, n=3 or 4 in each group.
dependent inhibition of ex vivo platelet aggregation. DMP 728 demonstrated reversible effects on bleeding time prolongation while maintaining maximal inhibition of platelet aggregation.32 The antiplatelet effects of DMP 728 were shown at extremely low dose levels. It was also shown that the antagonism of the platelet GPIIb/IIIa receptors in anesthetized dogs by DMP 728 had no effects on any of the different hemodynamic parameters (data not shown) or platelet counts over the wide range of doses administered. No spontaneous bleeding was observed at any sites other than the confined sites of bleeding time measurements. These data suggest that DMP 728 is a potent and systemically active antiplatelet agent with high affinity for the platelet GPIIb/IIIa receptors. Additionally, the high affinity and specificity of DMP 728 for platelet GPIIb/IIIa might explain the observed optimal efficacy/safety ratios. DMP 728 demonstrated antithrombotic efficacy in various models of in vitro and in vivo arterial thrombosis. In an in vitro isolated rabbit aorta perfused with epinephrine-primed human blood and injured by external clamping or with balloon-induced intimal wall injury, DMP 728 maximally inhibited both platelet deposition and arterial flow reduction at 0.1 μmol/L. Neither aspirin nor ticlopidine at concentrations up to 1 mmol/L was effective in this model. In an unstable angina—type model (the Folts model in dogs), DMP 728 inhibited CFR with an ED50 of 0.005 mg/kg IV. In the Folts model, DMP 728 (0.01 mg/kg IV bolus) demonstrated maximal efficacy in inhibiting ex vivo platelet aggregation, totally preventing CFR and maintaining coronary flow and coronary arterial patency. These studies suggest the potential of DMP 728 in unstable angina. In contrast, aspirin (0.5 to 5.0 mg/kg IV) was shown to be marginally effective in this model and ineffective against epinephrine-reinduced CFR.23 Hence, it is anticipated that DMP 728 might be more effective than aspirin in unstable angina, since platelet activation could be reinduced after elevation of catecholamines, rupture of atherosclerotic plaques, or progression of atherosclerotic lesions. Furthermore, maximal efficacy against epinephrine-reinduced CFR was demonstrated with other GPIIb/IIIa antagonists such as SK&F 106760.23 These results suggest greater efficacy for GPIIb/IIIa antagonists compared with aspirin.

In the femoral artery thrombosis and rethrombosis model in dogs, DMP 728 (1.00 mg/kg IV) demonstrated maximal antithrombotic efficacy in electrolytically induced femoral artery thrombosis and rethrombosis after thrombolysis with either TPA or SK. Despite the increased thrombogenic stimulus as demonstrated in the electrolytically induced arterial thrombosis in the femoral artery, DMP 728 shortened the time to reperfusion when given in conjunction with either SK or TPA and was also capable of limiting further platelet deposition at the site of the damage. However, it remains to be determined in a clinical setting how long after thrombolysis platelet inhibition must be sustained to render injured vessel and resid-

![Graph showing antithrombotic effects of DMP 728 in coronary artery thrombosis in dogs (Folts model). Top, Representative illustration of the effects of DMP 728 (0.005 to 0.01 mg/kg IV bolus) on mean arterial blood pressure, heart rate, mean left circumflex artery (LCx) flow, or phasic LCx flow. Although DMP 728 did not affect the different hemodynamic parameters, it maintained mean basal and phasic LCx flow in anesthetized dogs. Bottom, Effects of DMP 728 (0.005 to 0.01 mg/kg IV bolus) on the mean percent incidence of cyclic flow reduction (CFR) in the LCx of anesthetized dogs. Data represent mean±SEM, n=4 to 11 animals in each group.](http://circ.ahajournals.org/)

**Fig 5.** Graphs showing antithrombotic effects of DMP 728 in coronary artery thrombosis in dogs (Folts model). Top, Representative illustration of the effects of DMP 728 (0.005 to 0.01 mg/kg IV bolus) on mean arterial blood pressure, heart rate, mean left circumflex artery (LCx) flow, or phasic LCx flow. Although DMP 728 did not affect the different hemodynamic parameters, it maintained mean basal and phasic LCx flow in anesthetized dogs. Bottom, Effects of DMP 728 (0.005 to 0.01 mg/kg IV bolus) on the mean percent incidence of cyclic flow reduction (CFR) in the LCx of anesthetized dogs. Data represent mean±SEM, n=4 to 11 animals in each group.

**TABLE 4. Effects of DMP 728 on Time to Occlusion, Time to Reocclusion, Time to Reperfusion, and Residual Thrombus Weights in an Electrolytically Induced Femoral Artery Thrombosis Model in Dogs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time to Occlusion, min</th>
<th>Time to Reocclusion, min</th>
<th>Time to Reperfusion, min</th>
<th>Thrombus Weight, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline/SK</td>
<td>104±11</td>
<td>42±10</td>
<td>60.0±20</td>
<td>32.0±8.0</td>
</tr>
<tr>
<td>2. DMP 728/SK</td>
<td>96±8</td>
<td>&gt;240*</td>
<td>11.4±3.3*</td>
<td>8.5±2.4*</td>
</tr>
<tr>
<td>3. Saline/TPA</td>
<td>109±12</td>
<td>32±9</td>
<td>ND</td>
<td>48.3±4.9</td>
</tr>
<tr>
<td>4. DMP 728/TPA</td>
<td>101±14</td>
<td>&gt;240*</td>
<td>ND</td>
<td>9.1±2.5*</td>
</tr>
</tbody>
</table>

DMP 728 administered at 1.0 mg/kg IV bolus 30 minutes before streptokinase (SK) or tissue-type plasminogen activator (TPA). Time to reperfusion was not determined (ND) in groups 3 and 4 because of the high frequency of an oscillatory flow or CFRs in group 3. The range of time to reperfusion in group 3 was 20 to 40 minutes, compared with 10 to 30 minutes in group 4. Data represent mean±SEM, n=6 animals in each group.

*P<.01 compared with the respective saline group.
ual thrombus mass nonthrombogenic. These studies indicate that DMP 728 is a potent antithrombotic agent in preventing thrombus formation or rethrombosis after thrombolysis. These data also suggest that DMP 728, a low-molecular-weight GPIIb/IIIa receptor antagonist, may have therapeutic potential as an effective antithrombotic agent in coronary and peripheral artery thromboembolic disorders.

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Antiplatelet and antithrombotic efficacy of DMP 728, a novel platelet GPIIb/IIIa receptor antagonist.
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