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

Shaker A. Mousa, PhD; Jeffery M. Bozarth, AS; Mark S. Forsythe, BS; Sharon M. Jackson, MS; Andrew Leamy, AS; Mark M. Diemer, MS; Ram P. Kapil, PhD; Robert M. Knabb, PhD; Michael C. Mayo, BS; Sandra K. Pierce, AS; William F. De Grado, PhD; Martin J. Thoolen, PhD; Thomas M. Reilly, PhD

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, DMP 728 demonstrated a more rapid antithrombotic efficacy at 0.01 mg/kg IV bolus with an ED50 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 electrocytologically 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 pathological conditions, including cardiovascular and cerebrovascular thromboembolic disorders such as unstable angina, myocardial infarction, transient ischemic attack, stroke, and atherosclerosis.1-5 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.6 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.7-9

Current antiplatelet drugs are mainly effective against one of the many platelet activators. These drugs include aspirin, which blocks cyclooxygenases10; ticlopidine, which acts against ADP11; thromboxane A2 synthetase inhibitors or receptor antagonists, which act against thromboxane A212; and hirudin, which acts against thrombin.13 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.14 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.12,15

The platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa) has recently been identified as the final common pathway for all agonists.16,17 The binding of adhesive proteins, such as fibrinogen, to GPIIb/IIIa causes plate-
Fig 1. Chemical structure of DMP 728. DMP 728 is the (cyclic[N-[2-aminobutyryl]-N-(2-methyl-L-arginyl)-L-aspartyl-3-amino-
 methyl-benzoic acid] methanesulfonic acid salt), which has the following physicochemical properties: molecular weight, 656.7; purity, >99.0% as determined by a high-performance liquid chromatographic method; solubility, soluble in water at >150 mg/mL; stability, stable in solution at pH 7.4 and 4.5 at room temperature.

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) (5×10^6 platelets/mL) 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.25 with some modifications as described below. Human PRP (h-PRP) was applied to a sepharose column to prepare human gel-purified platelets (h-GP). Aliquots of h-GP (5×10^6 platelets/mL) along with 1 mmol/L calcium chloride were added to removable 96-well plates, 125I-fibrinogen (26.5 μCi/mg) was added, and the h-GP 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 bound 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, enterokinases, proteases, and peptidases was determined as well. [3H]DMP 728 (24.3 Ci/mmol) with a chemical purity of 99% was incubated in the presence or absence of the previously described microsomal, nonmicrosomal, or proteolytic enzymes. 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 high-performance liquid chromatography (HPLC) with on-line radiochemical detection. The metabolic viability of the different enzyme systems 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.

Methods

Reagents

ADP, collagen from calf skin, epinephrine bitartrate, and other reagents used in these studies were obtained from Sigma Chemical Co (St Louis, Mo). Tissue-type plasminogen activator was purchased from Genentech Inc (San Francisco, Calif). Streptokinase was obtained from Hoechst-Roussel Pharmaceutical Inc (Somerville, NJ). Arachidonate was purchased from Nu Chek Prep (Elkaidan, Minn). 125I-Fibrinogen (26.5 μCi/mg) was obtained from Du Pont NEN (Boston, Mass). DMP 728 (cyclic[N-[2-aminobutyryl]-N-(2-methyl-L-arginyl)-
 glycol-L-aspartyl-3-aminomethyl-benzoic acid] methanesulfonic acid salt) and RGDS were synthesized at the DuPont Merck Pharmaceutical Co (Wilmington, Del).
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 proteins 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 Simplette 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 (Couler Diagnostics), and platelet number was determined with a Couler Counter (Couler 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 7 H₂O 1.2, CaCl₂ 2 H₂O 0.66, 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 (10⁻¹⁷ 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.²⁸,²⁷ 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 CFRs 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. 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 intima of 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. 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·min−1 until lysis occurred; SK was administered at 10 000 U/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.

**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-GP IIb-IIIa antagonist 125I-fibrinogen binding assay, DMP 728 inhibited 125I-fibrinogen binding to activated h-GP 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 after thrombolysis as 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.

**Table 1. In Vitro Antiplatelet Efficacy of DMP 728 in Platelets From Various Species**

<table>
<thead>
<tr>
<th>Species</th>
<th>PRP Aggregation</th>
<th>125I-Fibrinogen Binding</th>
</tr>
</thead>
<tbody>
<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>
</tr>
</tbody>
</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 platelet discs 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.
(ICso=2 to 5 nmol/L) in inhibiting 125I-fibrinogen binding to h-GP 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 ICso of 10 to 30 μmol/L. DMP 728 not only prevented 125I-fibrinogen binding but also displaced 125I-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 Anti thrombotic 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 anti thrombotic effects of other standard antiplatelet agents, including aspirin and ticlopidine as well as the GPiIIb/IIIa prototype antagonist RGDS, were evaluated. RGDS at 1.0 mmol/L demonstrated 100% anti thrombotic efficacy in reducing CFR, compared with maximal anti thrombotic efficacy with DMP 728 at 0.1 μmol/L. In contrast, neither aspirin nor ticlopidine showed any distinct anti thrombotic efficacy at concentrations up to 1.0 mmol/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 Anti thrombotic Efficacy in Dogs

Coronary Artery Thrombosis in Dogs (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. 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 anti thrombotic 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 anti thrombotic efficacies at 0.01 mg/kg IV bolus in anesthetized dogs (Table 2 and Fig 5). The onset for the anti thrombotic effects of DMP 728 was immediate, with a dose-dependent duration of action.

Femoral Artery Restenosis in Dogs (Electrolytic Injury Model). A total of 24 mongrel dogs, divided into four groups, were entered into this arm of the study. There were no statistically significant differences among the groups with regard to the time to occlusion (Table 4). The effects of DMP 728 on the incidence of
TABLE 2. Antiplatelet Effects of Intravenous DMP 728 in Anesthetized Mongrel Dogs

<table>
<thead>
<tr>
<th>Dose Levels, mg/kg IV bolus</th>
<th>Time, min</th>
<th>Inhibition PA,* %</th>
<th>Bleeding Levels, t, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>27</td>
<td>3.3 7±1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>05</td>
<td>2.9 3±1</td>
</tr>
<tr>
<td>0.005</td>
<td>5</td>
<td>92±4</td>
<td>7.0 37±5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>55±5</td>
<td>3.2 18±4</td>
</tr>
<tr>
<td>0.01</td>
<td>5</td>
<td>100±0</td>
<td>&gt;15 86±6</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>96±4</td>
<td>4.5 42±8</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>81±4</td>
<td>4.2 31±5</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>80±5</td>
<td>29±3</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>62±6</td>
<td>3.5 15±3</td>
</tr>
<tr>
<td>0.1</td>
<td>5</td>
<td>97±4</td>
<td>359±32</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>98±5</td>
<td>&gt;15 200±6</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>95±7</td>
<td>131±4</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>97±0</td>
<td>87±4</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>100±0</td>
<td>&gt;15 21±2</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>65±11</td>
<td>10±3</td>
</tr>
<tr>
<td>1.0</td>
<td>5</td>
<td>100±0</td>
<td>4015±222</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>100±0</td>
<td>&gt;15 1624±100</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>100±0</td>
<td>878±70</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>100±0</td>
<td>375±20</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>100±0</td>
<td>&gt;15 230±15</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>100±0</td>
<td>93±2</td>
</tr>
</tbody>
</table>

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>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg/kg IV bolus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>324±48</td>
<td>5.1±0.2</td>
<td>35.0±2.5</td>
</tr>
<tr>
<td>60 min</td>
<td>292±38</td>
<td>5.4±0.3</td>
<td>35.9±2.5</td>
</tr>
<tr>
<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 $42\pm10$
and $32\pm9$ minutes after lysis, respectively (Fig 6 and
Table 4). Interestingly, a steady and stable arterial flow
in the DMP 728-treated group was noted, com-
pared 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% pre-
vention 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 ad-
ministered 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 com-
mon pathway for all agonist-induced platelet aggre-
gate formation.$^6,^7,^17$ The binding of fibrinogen is me-
diated 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 mono-
clonal 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 advantageous 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, re-
respectively) in inhibiting platelet aggregation in human
PRP.$^{30}$ DMP 728 demonstrated high affinity and simi-
lar potency in inhibiting platelet aggregation regard-
less of the agonist used.$^{31}$ Additionally, DMP 728
demonstrated a high degree of selectivity toward the
platelet GPIIb/IIIa receptors compared with the
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. Addi-
tionally, a minimal and perhaps insignificant level
(15% to 25%) of plasma protein binding was dem-
strated with DMP 728. Intravenous administration of
DMP 728 in anesthetized dogs produced a dose-
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.
vascular 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.

References


Antiplatelet and antithrombotic efficacy of DMP 728, a novel platelet GPIIb/IIIa receptor antagonist.
S A Mousa, J M Bozarth, M S Forsythe, S M Jackson, A Leamy, M M Diemer, R P Kapil, R M Knabb, M C Mayo and S K Pierce

_Circulation._ 1994;89:3-12
doi: 10.1161/01.CIR.89.1.3

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/1/3