Coronary Thrombolysis With Desmodus Salivary Plasminogen Activator in Dogs

Fast and Persistent Recanalization by Intravenous Bolus Administration

Werner Witt, PhD; Bernhard Maass; Berthold Baldus, MS; Michael Hildebrand, PhD; Peter Donner, PhD; Wolf-Dieter Schleuning, MD

Background  DSPA (Desmodus salivary plasminogen activator) is a new thrombolytic agent corresponding to a natural plasminogen activator discovered in the saliva of the vampire bat Desmodus rotundus. Compared with tissue plasminogen activator (TPA), DSPA, produced in a recombinant cell line, is more fibrin cofactor dependent than TPA.

Methods and Results  The thrombolytic properties of DSPA and TPA were compared in a canine model of copper-coinduced coronary thrombosis. All dogs received heparin 200 IU/kg IV and SC. Whereas controls did not reperfuse within 180 minutes (none of six), intravenous bolus administration of DSPA at 25, 50, and 100 μg/kg resulted in a 100% incidence (6 of 6) of recanalization within 37, 23, and 18 minutes, respectively. TPA at 63 and 125 μg/kg reopened the coronaries in 33% (two of six) and 50% (three of six) of cases within 40 minutes. Eighty-three percent (5 of 6) of the arteries were still patent 3 hours after 50 and 100 μg/kg DSPA, whereas only 20% (one of five) of all coronaries originally recanalized with both doses of TPA were still open at 2 hours. Plasma levels of α2-antiplasmin decreased significantly only with 125 μg/kg TPA. The clearance of DSPA (2.3 to 3.5 mL·min⁻¹·kg⁻¹) was lower compared with TPA (11.4 to 20 mL·min⁻¹·kg⁻¹) due to a prolonged terminal half-life.

Conclusions  In a canine coronary thrombosis model, DSPA exhibited higher potency and recanalized coronary arteries faster and with a lower incidence of reocclusion than TPA. Its properties may translate into a higher efficacy in patients compared with available thrombolytic agents. The long half-life of DSPA may allow for single bolus administration in the treatment of acute myocardial infarction. (Circulation. 1994; 90:421-426.)

Key Words  reocclusion · tissue plasminogen activator · thrombolysis · Desmodus salivary plasminogen activator

The results of the GUSTO (Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries) trial appear to favor an accelerated tissue plasminogen activator (TPA) regimen over streptokinase in the treatment of myocardial infarction. A significantly lower total mortality was associated with the fast TPA regimen. GUSTO also demonstrated that early recanalization was essential to the survival benefit seen with TPA, and, in general, the earlier thrombolytic treatment was initiated, the better its results. TPA, a drug with partial fibrin dependency, thus has been shown to be superior to streptokinase, a non–fibrin-dependent thrombolytic. To elucidate the significant differences between the two agents, however, the enrollment of large numbers of patients was required, and, even with the accelerated, front-loading TPA regimen used, sufficient patency (TIMI grade 3) at 90 minutes was observed in only 53% of the patients.

GUSTO nevertheless suggests that an even more aggressive bolus administration of a fibrin-dependent thrombolytic agent may further improve early and complete recanalization with a possible further increase in total survival in myocardial infarction. However, TPA, because of its short half-life and its limited fibrin specificity, may not be the ideal candidate for this purpose. The administration of high doses of TPA to overcome its short half-life is associated with the induction of severe plasminemia, coagulopathy, intracerebral hemorrhage, and stroke.2-4 Depletion of circulating (extrinsic) plasminogen with high plasma levels of TPA, a consequence of limited fibrin specificity, would also lower efficacy of TPA on the clot surface through an effect termed “plasminogen steal.”5,6 Hence, although GUSTO demonstrated the superiority of partially fibrin-dependent clot lysis, the available thrombolytic agents may not achieve the maximum possible benefit of therapeutic thrombolysis.

The ongoing search for new thrombolytic agents has consequently focused on mutants of TPA, the major goals being the improvement of fibrin cofactor dependence without losing specific activity and the prolongation of half-life compared with the wild type.7-9 The most significant improvement in all the above properties, however, was achieved by the cloning and expression of natural plasminogen activators from the saliva of the vampire bat Desmodus rotundus.10,11 The two high-molecular-weight forms of this family, DSPAα₁ (referred

Received February 18, 1994; revision accepted March 24, 1994.
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to as DSPA in this report) and DSPA$_\alpha$ (also named bat-PA or bat-PA[H]), exhibit a specific activity in vitro equal to or higher than that of TPA, a relative plasminogen activator inhibitor-1 (PAI-1) resistance, and a greatly enhanced fibrin specificity with a strict requirement for polymeric fibrin as a cofactor.$^{12-14}$ In animal models of thrombolysis, DSPA$_\alpha$ and DSPA$_\beta$ are superior to TPA in terms of potency, half-life, and clearance.$^{15-17}$ Interestingly, the fibrin cofactor requirement of DSPA$_\alpha$ and DSPA$_\beta$, which both bind to fibrin, may not solely depend on fibrin binding, as the two smaller forms, DSPA$\beta$ (bat-PA[I]) and DSPA$\gamma$ (bat-PA[L]), are also fibrin dependent but lack fibrin affinity.$^{10}$

The present study investigates the pharmacokinetic and thrombolytic properties of DSPA compared with TPA after intravenous bolus administration in a canine model of coronary artery thrombosis. The thrombogenic stimulus in this model is a copper coil introduced into the coronary artery, which usually results in rapid occlusion of arteries by platelet-rich thrombi.$^{18}$ Successful recanalization by thrombolytic treatment in our model is verified angiographically and is followed by a 100% reocclusion rate for both TPA and streptokinase infused in the absence of heparin, as we have previously shown.$^{19}$ In the present investigation, bolus administration of TPA resulted in a partial reperfusion in some dogs only, and almost all arteries initially recanalized with TPA still reoccluded despite using adjunctive heparin. In contrast, equimolar doses of DSPA in the presence of heparin reopened all coronaries, with most of them being still patent 180 minutes after dosing. Compared with TPA, the reperfusion times achieved with DSPA were shorter, $\alpha_2$-antiplasmin levels did not decrease, and the clearance of DSPA was five to nine times slower because of a massively extended terminal half-life.

**Methods**

**Animal Model**

Thirty-six male beagles (10.1 to 17.6 kg body wt) were anesthetized by intravenous injection of 20 mg/kg thiopental sodium. Anesthesia was maintained with tramadol-HCl, 2 mg/kg IV bolus and 0.5 mg/kg per hour IV infusion, accompanied by artificial ventilation with 20% $O_2$-80% N$_2$O. To allow positive-pressure ventilation, 0.33 mg/kg IV hexobarbital bromide was given, with additional doses of 0.17 mg/kg every 2 hours. Coronary thrombosis was induced by placement of a copper coil into a branch of the left circumflex artery (a. marginalis) as described previously.$^{19,20}$ The copper coil (three turns; diameter, 2.5 mm; length, 3.5 mm; wire, 0.5 mm) was positioned under roentgenographic control. The coronary perfusion status was assessed by repeated angiography (every 5 minutes until, every 10th minute after first reperfusion) using Urografin (Schering AG). When coronary occlusion accompanied by typical ECG signs (ST-elevation) was observed, 60 minutes were allowed for thrombus growth and stabilization before all animals received 200 U/kg IV plus 200 U/kg SC heparin (Liquemin, Hoffmann-La Roche) (repeated after 2 hours) followed by injection of the thrombolytics.

The animals were randomly assigned to receive either 25, 50, or 100 $\mu$g/kg DSPA (Schering AG and Berlex Biosciences), 63 or 125 $\mu$g/kg TPA (Actilyse), or 1 mL/kg control buffer by intravenous bolus administration over 2 minutes through a femoral vein catheter. As parameters of thrombolytic efficacy, we determined the time to initial recanalization, the recanalization incidence (number of dogs with at least one period of reopening of the artery), total patency time (assuming persistence of patency occlusion between two succeeding angiograms), and the patency incidence at the end of the observation period (180 minutes after dosing).

Blood samples were taken before administration of the thrombolytics at the end of the occlusion period and 2, 4, 8, 16, 32, 64, 120, and 180 minutes after dosing.

This study conforms to the "Guiding Principles in the Care and Use of Animals" for research of the American Physiological Society and are in accordance with the German "Animal Protection Act" of August 18, 1986 (amended February 17, 1993).

**Hemostasis Factors**

Femoral vein whole blood samples were anticoagulated by addition of 3.8% trisodium citrate solution (9+1 vol+vol). Plasma obtained was supplemented with PPACK (Calbiochem; 1 $\mu$mol/L final concentration) and kept at −80°C to prevent in vitro fibrinogen degradation.

Plasma fibrinogen was measured as clottable protein. Plasminogen and $\alpha_2$-antiplasmin were determined using chromogenic substrate assays (S-2251, KabiVitrum). $\alpha_2$-Antiplasmin was expressed as percentage of that in pooled plasma from male beagles.

**Plasminogen Activator Antigen**

TPA antigen levels were determined by a commercially available ELISA kit (Biopool). DSPA$_\alpha$ antigen was measured by our own ELISA as previously described.$^{16}$

**Pharmacokinetics**

Evaluation was performed by using TOPFIT, version 2.0, applying an open two-compartment model to both TPA and DSPA, individually taking into account the different doses.$^{21}$ Some animals were excluded from the analysis because of sampling or assay errors as indicated (n=4 to 6).

**Statistics**

Data are presented as either mean±SEM or median±SEM. The percentage of decrease in $\alpha_2$-antiplasmin values and total patency times in verum groups were compared with controls by ANOVA and Dunnett's $t$ test ($P$<.05 per comparison). Comparisons of incidences were conducted with Fisher's exact test ($P$<.05).

**Results**

**Efficacy of DSPA and TPA**

After introduction of the copper coil, all coronary arteries occluded in a mean of 17 to 23 minutes (Table 1). In none of the control dogs, treated with heparin only, did spontaneous recanalization occur. TPA at 63 and 125 $\mu$g/kg resulted in reperfusion of two of six and three of six arteries, respectively, which was not significantly different from controls, in a mean of 40 minutes. DSPA, however, was already fully effective at the lowest dose tested (25 $\mu$g/kg), eliciting recanalization in six of six dogs within 37 minutes after dosing. The two higher doses of DSPA, 50 and 100 $\mu$g/kg, being equimolar to the doses of TPA used in our experiments, were also 100% effective and resulted in a dose-dependent lowering of the time to recanalization to 23 and 18 minutes after dosing, respectively.

The five arteries recanalized with either dose of TPA remained open for 5 to 140 minutes (range) within our observation period of 180 minutes after dosing, whereas only one of them was still patent at 180 minutes (Fig 1 and Table 2). DSPA mediated a dose-dependent increase in the total patency time from 23 to 155 and 162
minutes (medians) for 25, 50, and 100 μg/kg, respectively. Five of six dogs with reperfusion upon 25 μg/kg DSPA had patency times lasting only 10 to 40 minutes, and all arteries in this dose group reoccluded by 180 minutes after dosing. The median total patency times after 50 and 100 μg/kg DSPA differed only slightly (155 and 163 minutes, respectively), and 83% (five of six) of the arteries in both groups were still open at 180 minutes.

**Effects on Hemostasis**

Neither fibrinogen nor plasminogen levels decreased compared with controls with any dose of either TPA or DSPA tested (data not shown). α2-Antiplasmin, however, was significantly lower than in controls at 180 minutes after administration of 125 μg/kg TPA (Table 2).

**Pharmacokinetics**

An optimal fit was achieved by applying an open two-compartment model on the disposition profile of DSPA antigen in dogs. For comparison, TPA also was analyzed assuming a two-compartment disposition, for which we also obtained an excellent fit. The most striking difference between the elimination curves for DSPA and TPA after intravenous bolus administration was the prolonged terminal half-life of DSPA with mean plasma levels 3 hours after dosing of 14, 43, and 82 ng/mL after 25, 50, and 100 μg/kg DSPA, respectively (Fig 2). Whereas initial half-lives were quite similar for both plasminogen activators, DSPA had a terminal half-life of 188.9 to 199.2 minutes compared with 4.6 to 8.2 minutes for TPA, with 96% to 98% of DSPA cleared in this phase (Table 3). This resulted in a pronounced prolongation of the mean residence time of DSPA compared with TPA and a greatly reduced total clearance of the bat plasminogen activator. The clearance rate of DSPA was 4.8- to 8.7-fold lower than that of equimolar doses of TPA.

**Discussion**

We have demonstrated that DSPA rapidly recanalized occluded coronary arteries in the copper coil model in dogs after single intravenous bolus administration. TPA by single bolus administration of up to 125 μg/kg did not result in a reperfusion incidence above 50%. Regardless of heparinization, reoclusion was prevalent after restoration of blood flow with all doses of TPA and the lowest dose of DSPA used in our experiments. These results indicate a rather poor efficacy of heparin in this dog model for prevention of early reoclusion, supporting the need for better adjunctive therapy with TPA. With respect to DSPA, rather low plasma levels of approximately 40 ng/mL may have successfully maintained the patency of reperfused coronaries throughout our observation period of 180 minutes by inhibiting rethrombosis. Similar antithrombotic effects have been observed in dogs treated with subthrombolytic doses of TPA.22 On the other hand, the low rate of reoclusion with DSPA also may indicate a more complete lysis with the bat plasminogen activator. Indeed, the superior efficacy of DSPA compared with TPA is striking and probably best reflected in total patency time.

With regard to potency, DSPA bolus administration may be more than five times as potent as bolus TPA, as 25 μg/kg of the bat fibrinolytic is already superior to 125 μg/kg of TPA both in respect to reperfusion incidence and total patency time. If one expresses in vivo specific activity as reperfusion incidence or total patency time

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**Table 1. Occlusion of Coronary Arteries and Recanalization After Intravenous Bolus Treatment With the Bat Plasminogen Activator DSPA and TPA in Dogs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Occlusion Time, min</th>
<th>Incidence of and Time to Recanalization</th>
<th>Ratio</th>
<th>%</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23±4</td>
<td></td>
<td>0:6</td>
<td>0</td>
<td>. . .</td>
</tr>
<tr>
<td>DSPA 25 μg/kg</td>
<td>17±3</td>
<td></td>
<td>6:6</td>
<td>100*</td>
<td>37±13</td>
</tr>
<tr>
<td>DSPA 50 μg/kg</td>
<td>28±7</td>
<td></td>
<td>6:6</td>
<td>100*</td>
<td>23±3</td>
</tr>
<tr>
<td>DSPA 100 μg/kg</td>
<td>22±2</td>
<td></td>
<td>6:6</td>
<td>100*</td>
<td>18±4</td>
</tr>
<tr>
<td>TPA 50 μg/kg</td>
<td>18±5</td>
<td></td>
<td>2:6</td>
<td>33</td>
<td>40±0</td>
</tr>
<tr>
<td>TPA 125 μg/kg</td>
<td>20±4</td>
<td></td>
<td>3:6</td>
<td>50</td>
<td>40±10</td>
</tr>
</tbody>
</table>

DSPA indicates Desmodus salivary plasminogen activator; TPA, tissue plasminogen activator.

Oclusion and recanalization times represent mean±SEM.

*P<.05 vs control by Fisher's exact test.

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Fig 1. Patency profiles of coronary arteries in individual dogs after intravenous bolus administration of 25, 50, or 100 μg/kg Desmodus salivary plasminogen activator (DSPA), 63 or 125 μg/kg tissue plasminogen activator (TPA), or solvent (control). Each stacked bar represents the angiographically defined patency status of a single dog coronary artery throughout our observation period of 180 minutes after dosing. Solid bars reflect periods of coronary occlusion; open bars depict periods of arterial reflow.
per area under the curve applied, then DSPA also may have a higher in vivo specific activity than TPA in dogs. The 25 \( \mu g/kg \) DSPA and 125 \( \mu g/kg \) TPA roughly result in the same area under the curve, whereas DSPA is clearly more effective at this dose.

Two other plasminogen activators have been described with properties similar to DSPA: (1) the Desmodus plasminogen activator DSPA\(_{25}\) (bat-PA) and (2) TNK (T103N, N117Q, KHRR296-299AAA), a mutant form of TPA representing the most successful product of various efforts directed toward improving specificity and half-life of wild-type TPA. Two studies in canine arterial thrombosis models are available comparing bolus administrations of either DSPA\(_{25}\) or TNK with TPA.\(^{25,26}\) In both studies, the different doses of TPA used achieved the same recanalization incidence of 50%, as in our present study. DSPA\(_{25}\) administered at a dose equimolar to TPA achieved a recanalization incidence of 88%; TNK applied at a dose one third that of TPA resulted in an arterial recanalization incidence of 92% in the respective models used. Despite these good although lower efficacies compared with DSPA in our model, reocclusion remained a significant problem in both studies. With DSPA\(_{25}\) and TPA, reocclusion was 100% at 4 hours after dosing; similar total patency times achieved with TNK and TPA in the respective study indicate that TNK may be at least as prone to rethrombosis as TPA.

A pronounced plasminemia was not observed with either TPA or DSPA in the present study. Plasminogen and fibrinogen levels did not change compared with controls. On the other hand, \( \alpha\_2\)-antiplasmin values were measured in normal dogs. It is greatly enhanced, which suggests that DSPA does not activate extrinsic plasminogen because of its greater fibrin dependence. DSPA therefore is a plasminogen-sparing thrombolytic, resulting in the most economic use of the available plasminogen pool, thus also avoiding development of plasminemia and consumption coagulopathy associated with enhanced bleeding episodes at higher doses of TPA.

The pharmacokinetic properties of DSPA may allow its use in myocardial infarction by single bolus administration. This should result in a more rapid reperfusion at lower doses compared with TPA and a reduced risk of reocclusion. The half-life of DSPA in dogs is greatly prolonged compared with TPA, with the mean residence time being about 40 to 50 times longer than that of TPA. DSPA\(_{25}\) (bat-PA) displayed a mean residence time of sixfold longer than that of TPA and a clearance only slightly below that of TPA in dogs.\(^{23}\) Indeed, this may be the reason why DSPA\(_{25}\) appears to be more prone to reocclusion than DSPA. The clearance of DSPA is about five to nine times slower than that of TPA, the TPA mutant analog TNK being cleared about half as fast as the wild type.\(^{24}\) Again, the faster clearance of TNK over TPA may translate into a minor advantage of TNK over TPA only in both relative potency and total patency (including reocclusion). BM 06.022, another mutant consisting of the kringle 2 and protease domains of human TPA, has a half-life of 13 minutes and a clearance of 5.1 mL·min\(^{-1}\)·kg\(^{-1}\) in dogs.\(^{26}\) It is being pursued in clinical trials as a less costly

### Table 2. Terminal Patency, Total Patency, and \( \alpha\_2\)-Antiplasmin Levels 180 Minutes After Dosing of Intravenous Bolus DSPA and TPA in the Canine, Copper Coil Model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ratio</th>
<th>%</th>
<th>Total Patency Time, min</th>
<th>( \alpha_2)-Antiplasmin, % Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0:6</td>
<td>0</td>
<td>0</td>
<td>-2±3</td>
</tr>
<tr>
<td>DSPA 25 ( \mu g/kg )</td>
<td>0:6</td>
<td>0</td>
<td>23±40†</td>
<td>0±2</td>
</tr>
<tr>
<td>DSPA 50 ( \mu g/kg )</td>
<td>5:6</td>
<td>83</td>
<td>155±25†</td>
<td>2±1</td>
</tr>
<tr>
<td>DSPA 100 ( \mu g/kg )</td>
<td>5:6</td>
<td>83</td>
<td>163±17†</td>
<td>3±2</td>
</tr>
<tr>
<td>TPA 63 ( \mu g/kg )</td>
<td>1:6</td>
<td>17</td>
<td>0±32</td>
<td>4±3</td>
</tr>
<tr>
<td>TPA 125 ( \mu g/kg )</td>
<td>0:6</td>
<td>0</td>
<td>3±40</td>
<td>10±4*</td>
</tr>
</tbody>
</table>

DSPA indicates Desmodus salivary plasminogen activator; TPA, tissue plasminogen activator. Total patency times are median±SEM and \( \alpha\_2\)-antiplasmin values are mean±SEM. \( ^{+}P<.05 \) vs control by one-way ANOVA on ranks and Dunnett's test.

![Fig 2. Line plot: Decay in plasma antigen levels measured by specific ELISAs of Desmodus salivary plasminogen activator (DSPA) and tissue plasminogen activator (TPA) after intravenous bolus administration of 25, 50, and 100 \( \mu g/kg \) DSPA and 63 and 125 \( \mu g/kg \) TPA in copper coil-induced coronary thrombosis in dogs. All values are means of \( n=4 \) to 6 dogs.](image-url)
Table 3. Pharmacokinetic Parameters of Intravenous Bolus Administrations of DSPA and TPA In Dogs With Copper Coll-Induced Coronary Artery Thrombosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DSPA 25 µg/kg</th>
<th>DSPA 50 µg/kg</th>
<th>DSPA 100 µg/kg</th>
<th>TPA 63 µg/kg</th>
<th>TPA 125 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1/2a1, min</td>
<td>0.9±0.0</td>
<td>0.8±0.0</td>
<td>0.8±0.0</td>
<td>1±0.8</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>t1/2a2, min</td>
<td>199.2±21.5</td>
<td>188.9±32.3</td>
<td>189.9±22.7</td>
<td>4.6±0.3</td>
<td>8.2±2.6</td>
</tr>
<tr>
<td>MRT, min</td>
<td>277</td>
<td>264</td>
<td>270</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Vss, mL/kg</td>
<td>95±11</td>
<td>73±9</td>
<td>125±10</td>
<td>27±3</td>
<td>66±22</td>
</tr>
<tr>
<td>AUC, ng·h/mL</td>
<td>127±16</td>
<td>370±44</td>
<td>729±63</td>
<td>92±4</td>
<td>124±24</td>
</tr>
<tr>
<td>AUC1/1, %</td>
<td>4±0</td>
<td>4±1</td>
<td>2±0</td>
<td>50±4</td>
<td>52±3</td>
</tr>
<tr>
<td>AUC2/2, %</td>
<td>96±0</td>
<td>96±1</td>
<td>98±0</td>
<td>50±4</td>
<td>48±3</td>
</tr>
<tr>
<td>Cl, mL·min⁻¹·kg⁻¹</td>
<td>3.5±0.4</td>
<td>2.4±0.3</td>
<td>2.3±0.2</td>
<td>11.4±0.5</td>
<td>20±4.3</td>
</tr>
</tbody>
</table>

DSPA indicates Desmodus salivary plasminogen activator; TPA, tissue plasminogen activator; t1/2a1 and t1/2a2, initial and terminal half-lives; MRT, mean residence time; Vss, volume of distribution in central compartment; AUC, area under plasma level vs time curves; and Cl, total plasma clearance. Pharmacokinetic parameters are mean±SEM of n=4 to 6 dogs.

References
Coronary thrombolysis with Desmodus salivary plasminogen activator in dogs. Fast and persistent recanalization by intravenous bolus administration.

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_Circulation_. 1994;90:421-426
doi: 10.1161/01.CIR.90.1.421

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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