Haemostatic safety of a unique recombinant plasmin molecule lacking kringles 2–5

Victor J. Marder1; Steve Manyak1; Theresa Gruber1; Abha Goyal1; Guillermo Moreno1; Jennifer Hunt2; John Bromirski2; Philip Scuderi2; Stephen R. Petteway, Jr2; Valery Novokhatny2

1David Geffen School of Medicine at UCLA, Los Angeles, California, USA; 2Talecris BioTherapeutics, Inc, Research Triangle Park, North Carolina, USA

Introduction

The potential of plasmin for treating human thrombotic disease was considered over 40 years ago (1), but until recently, only the plasminogen activators (PA) have been developed for clinical practice (2). While effective in dissolving thrombi and improving clinical outcome, PA are not ideal agents, primarily because of unavoidable risks for haemorrhagic complications (3). A new thrombolytic agent that achieves thrombolysis without causing haemorrhagic complications would allow for significant improvement in the treatment of serious thrombotic disease.

Unlike PA such as tissue-type PA (tPA), plasmin degrades fibrin directly, without requiring the precursor molecule, plasminogen (4). Plasmin is rapidly neutralised by α2-antiplasmin (5), so it is ineffective when administered intravenously. However, catheter delivery of plasmin promotes binding to and lysis of clots in experimental in vitro models (6) as well as in patients with thrombosed haemodialysis grafts (7). Theoretically, plasmin that enters the circulation after catheter delivery is neutralised by α2-antiplasmin, thereby avoiding bleeding, and animal studies confirm the haemostatic safety advantage of full-length, plasma-derived human plasmin over tPA (8, 9). These observations are under current study in a trial of full-length plasmin in patients with peripheral arterial graft occlusion (10) and acute ischaemic stroke (11).

Structural variants of plasmin produced by enzymatic degradation (12, 13) or recombinant technology (13–15) retain the light chain serine protease domain, alone or in combination with heavy chain serine protease domain, alone or in combination with heavy chain kringle domains (16). “Micro-plasmin” consists of the serine protease domain alone (13), and “mini-plasmin” contains kringle 5 connected to the serine protease domain (12). Recently, a novel plasmin molecule lacking kringle 2–5 (K2–5) plasmin, (ΔK2–5) plasmin, consisting of kringle 1 linked to the serine protease domain of plasmin. Agent was administered intravenously in a randomised, blinded manner in a rabbit model of fibrinolytic haemorrhage. A dose-related decrease in α2-antiplasmin, factor VIII, and fibrinogen followed administration of 1.8, 2.7, 3.7 and 4.6 mg/kg of (ΔK2–5) plasmin, with nadir fibrinogen concentrations of 65%, 40%, 30%, and 0% of initial levels, respectively. Mean primary bleeding time was undisturbed at 1.8 mg/kg (2.2 ± 0.7 minutes), minimally prolonged at 2.7 or 3.7 mg/kg (5 ± 2.9 and 4.4 ± 2.2 minutes), and prolonged at the purposefully toxic 4.6 mg/kg dose (12.8 ± 18.8 minutes). Equimolar amounts of (ΔK2–5) plasmin and full-length plasmin had equal in vitro clot lysis efficacy, but in the bleeding model, (ΔK2–5) plasmin showed better haemostatic competency than full-length plasmin. This safety advantage may be explained by higher residual amounts of plasma fibrinogen in animals given (ΔK2–5) plasmin rather than full-length plasmin. We demonstrate that a unique plasmin molecule mutant, (ΔK2–5) plasmin, possesses an advantage in hemostatic safety over an equimolar amount of full-length plasmin. 

Summary

We previously demonstrated a significant margin of haemostatic safety for full-length plasmin in comparison with tissue plasminogen activator (t-PA). We now report studies that compare haemostatic safety of full-length plasmin with a novel recombinant plasmin derivative, (ΔK2–5) plasmin, consisting of kringle 1 linked to the serine protease domain of plasmin. Agent was administered intravenously in a randomised, blinded manner in a rabbit model of fibrinolytic haemorrhage. A dose-related decrease in α2-antiplasmin, factor VIII, and fibrinogen followed administration of 1.8, 2.7, 3.7 and 4.6 mg/kg of (ΔK2–5) plasmin, with nadir fibrinogen concentrations of 65%, 40%, 30%, and 0% of initial levels, respectively. Mean primary bleeding time was undisturbed at 1.8 mg/kg (2.2 ± 0.7 minutes), minimally prolonged at 2.7 or 3.7 mg/kg (5 ± 2.9 and 4.4 ± 2.2 minutes), and prolonged at the purposefully toxic 4.6 mg/kg dose (12.8 ± 18.8 minutes). Equimolar amounts of (ΔK2–5) plasmin and full-length plasmin had equal in vitro clot lysis efficacy, but in the bleeding model, (ΔK2–5) plasmin showed better haemostatic competency than full-length plasmin. This safety advantage may be explained by higher residual amounts of plasma fibrinogen in animals given (ΔK2–5) plasmin rather than full-length plasmin. We demonstrate that a unique recombinant plasmin molecule, (ΔK2–5) plasmin, possesses an advantage in hemostatic safety over an equimolar amount of full-length plasmin.

Keywords

Plasmin, recombinant derivative, haemostasis

Correspondence to:
Victor J. Marder, MD
David Geffen School of Medicine at UCLA
1000 Veteran Ave., Room A3–29 Rehab Bldg
Los Angeles, CA 90095–1795, USA
Tel.: +1 310 825 4469, Fax: +1 310 825 0914
E-mail: vmarder@mednet.ucla.edu

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However, no information is yet available which assesses the clot-dissolving efficacy and especially the in vivo haemostatic safety of Δ (K2-K5) plasmin. To determine whether Δ (K2-K5) plasmin shares or even exceeds the striking safety profile of full-length plasmin (6, 7) and, therefore, whether it has potential for clinical use, we have compared Δ (K2-K5) plasmin with full-length, plasma-derived plasmin in a blinded prospective assessment of haemostatic safety in an established animal model of fibrinolytic bleeding (20).

Figure 1: In vitro clot lysis efficacy of equimolar amounts of full-length plasmin vs. Δ (K2-K5) plasmin. Clot lysis measured by change in clot weight after exposure to full-length plasmin (solid circles) and Δ (K2-K5) plasmin (solid squares) at the indicated final concentrations. Concentrations of Δ (K2-K5) plasmin are 46% that of full-length plasmin, so equimolar concentrations of each agent were compared. There was no difference in fibrinolytic efficacy between full-length and Δ (K2-K5) plasmin.

Materials and methods

Full-length plasmin (Plasmin, Human, TAL-05–00018, Talecris Biotherapeutics, Inc.) was purified from human plasma as described elsewhere (6). After lysine-Sepharose isolation of plasminogen from plasma fractions and activation of plasminogen to plasmin by streptokinase, plasmin was purified by affinity chromatography to remove residual plasminogen and streptokinase, filtered and exposed to acid pH to remove viruses and prions, formulated at acid pH to prevent autodegradation, and lyophilized. Preparation of Δ (K2-K5) plasmin was performed based on previously published methods (17). Briefly, a plasmid-expressing Δ (K2-K5) plasminogen was synthesised by Blue Heron Biotechnology (Bothell, WA, USA), and a sequence optimised for expression in Escherichia coli was inserted into pET 24b (+) vector (Novagen, EMD Biosciences, Merck KGaA, Darmstadt, Germany) between the Ndel and BamHI sites. Expressed and refolded protein was centrifuged to remove particulates and passed over lysine affinity resin (Lysine-Sepharose 4B; Amersham Biosciences, Piscataway, NJ, USA) to obtain ~40% yield of properly folded Δ (K2-K5) plasminogen. Recombinant Δ (K2-K5) plasminogen was activated to Δ (K2-K5) plasmin using urokinase immobilised on Sepharose 4B (GE Healthcare, Piscataway, NJ, USA), monitored by S-2251 activity, captured on benzamidine-Sepharose, eluted with low-pH buffer, and acidified at pH 3.6 with acetic acid-saline to prevent auto-degradation. When mixed with plasma or neutral pH buffer, Δ (K2-K5) plasmin re-activates and exerts activity. Agent purity was assessed by SDS-PAGE using the NuPAGE® Bis-Tris Pre-Cast 4–12% Gel system (Invitrogen, Carlsbad, CA, USA).

Recombinant Δ (K2-K5) plasmin migrates on SDS-PAGE as a single band in the 35–40 kDa region in non-reduced gels and as two single bands of 27 kDa and 15 kDa under reduced conditions, representing the kringle 1 and the serine protease domains, respectively (17). The observed molecular mass of Δ (K2-K5) plasmin is close to the expected value of 37,000 kDa based on the protein sequence and is approximately 46% of the molecular mass of full-length plasmin (81,000–82,000 kDa) (21). Full-length plasmin contains the same serine protease domain of 27 kDa and a band that migrates at 55 kDa, corresponding to kringle 5 of the heavy chain.

 Amidolytic activity of the final preparations was measured using H-D-Val-Leu-Lys-p-nitroanilide substrate (S2251, DiaPharma Group Inc, West Chester, OH, USA) (16). Specific activities were compared to an active-site titrated standard and expressed as % of active material. For this study, 93% potent full-length plasmin and 86% delta-plasmin were used. For estimation of in vitro fibrinolytic efficacy, rabbit blood was collected into 5-ml Vacutainer tubes (BD, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) containing 3.2% sodium citrate, clotted with calcium chloride (13 mM final concentration) and alpha-thrombin (0.75 IU final concentration) (Enzyme Research Laboratories, Inc, South Bend, IN, USA), incubated overnight at 37°C, washed with 0.9% sodium chloride, trimmed to 4 cm length x 0.7 cm width, weighed (mean start weight, 2 ± 0.2 g), and transferred into 3-ml
BD syringe barrels. Clots were injected with 1.0 ml test article into the central axis incubated for 2 hours at 37°C, then removed and weighed. Clot lysis was expressed as % weight reduction.

For comparative studies of full-length and Δ(K2–5) plasmin, we used molar-equivalent amounts of each agent. For example, a 17 μM infusate consisted of 1.4 mg/ml of full-length plasmin or 0.64 mg/ml of Δ(K2–5) plasmin, and a 43 μM infusate consisted of 3.5 mg/ml of full-length plasmin or 1.6 mg/ml of Δ(K2–5) plasmin.

Fibrinolytic haemorrhage was assessed using an established rabbit ear-puncture technique (20). The experimental protocol was approved by the University of California, Los Angeles, Institutional Animal Care and Use Committee (Chancellor's Animal Research Committee Protocol #2005–124–03B). New Zealand white rabbits (2.9–3.7 kg) (Charles River Laboratories, Inc, Wilmington, MA, or Harlan, Indianapolis, IN, both USA) were anaesthetised by intramuscular ketamine HCl (40 mg/kg) and xylazine (10 mg/kg) and maintained with 2.3% isoflurane (Phoenix Pharmaceuticals, Inc, St Joseph, MO, USA) by endotracheal tube (Mallinckrodt, Inc, St Louis, MO, USA) through which 100% oxygen was administered. The right external jugular vein was exposed surgically, catheterized with an 8-cm 4F 2-lumen catheter (Medcomp, Harleysville, PA, USA), and the appropriate volume of experimental agent, adjusted for body weight, was administered by infusion pump (Sage Instruments, Orion Research, Boston, MA, USA) over 60 minutes (min). Each rabbit received 1 of 30 coded samples (6 treatments, 5 animals/group), prospectively randomised and blinded to the observer.

Primary bleeding time (PBT) was determined after full-thickness ear punctures with a surgical blade (#11, Feather Safety Razor, Medical Division, Osaka, Japan) and recorded at 30-second intervals by absorption of blood onto 11-cm circular filter paper (Whatman International LTD, Maidstone, UK). PBT was performed at 30 min, 10 min, and immediately prior to infusion, and at 10, 30, 60, 70, 90, 120, 150, and 180 min after starting the infusion; all lesions were monitored for rebleeding. Animals were euthanised with intravenous pentobarbital sodium (5 mg in 1 ml) (Western Medical Supply, Inc., Arcadia, CA, USA).

Blood samples (4.5 ml) were collected through the jugular vein catheter at 30 min and just before infusion, and at 10, 60, 70, 90, 120, 150, and 180 min after starting the infusion; all lesions were monitored for rebleeding. Animals were euthanised with intravenous pentobarbital sodium (5 mg in 1 ml) (Western Medical Supply, Inc., Arcadia, CA, USA).

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Table 1: Primary bleeding times (PBT) with escalating doses of Δ(K2–5) plasmin.

<table>
<thead>
<tr>
<th>Agent</th>
<th>#</th>
<th>Prolonged PBTs (%)</th>
<th>Mean PBT (min ± SD)</th>
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<tr>
<td>Δ(K2–5) plasmin</td>
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<tr>
<td>1.8 mg/kg</td>
<td>40</td>
<td>0 (0%)</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>2.7 mg/kg</td>
<td>40</td>
<td>15 (38%)</td>
<td>5.0 ± 2.9</td>
</tr>
<tr>
<td>3.7 mg/kg</td>
<td>40</td>
<td>12 (30%)</td>
<td>4.4 ± 2.2</td>
</tr>
<tr>
<td>4.6 mg/kg</td>
<td>40</td>
<td>28 (70%)</td>
<td>12.8 ± 18.8</td>
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*Prolonged PBT defined as greater than 4.7 minutes (pre-treatment control + 3 SD, 2.3 ± 2.4 min). The number of prolonged PBTs with 2.7 or 3.7 mg/kg Δ(K2–5) plasmin was higher than with 1.8 mg/kg (X²=18.5 and 14.1, respectively), and higher with 4.6 mg/kg compared with 1.8, 2.7 or 3.7 mg/kg (X²=48, 11.4, 16.2, respectively). There was no significant difference between the 2.7 and 3.7 mg/kg dosages. *Mean PBT calculated for 8 sites after the start of agent infusion. Results with Δ(K2–5) plasmin were greater at 2.7, 3.7 or 4.6 mg/kg than at 1.8 mg/kg (P<0.0001, P<0.0001, P=0.0001), and greater at 4.6 than at 2.7 or 3.7 mg/kg (P=0.015, 0.005) using repeated measures ANOVA. There was no difference between the 2.7 and 3.7 mg/kg dosages (P=0.312).

Figure 2: Primary bleeding with escalating dosages of Δ(K2–5) plasmin.

Total dosages of 1.8, 2.7, 3.7, and 4.6 mg/kg of Δ(K2–5) plasmin were administered over 60 minutes. The pre-treatment primary bleeding time (PBT) was 2.3 ± 0.8 min (mean ± SD) for 90 determinations. Dashed lines are drawn at 2.3 + 2.4 = 4.7 min (mean plus 3 SD), arbitrarily taken as the upper limit of normal. Statistical comparisons are detailed in Table 1.
120, and 180 min after the start of infusion, and added to tubes containing 0.5 ml of 0.25 M sodium citrate anticoagulant. After centrifugation at 3,000 g at 4°C for 30 min, plasma was removed and tested immediately for factor VIII activity, fibrinogen concentration, and α₂-antiplasmin activity (22–24). The assay for α₂-antiplasmin is based on the facility of test sample (plasma) to neutralise added plasmin, using a chromogenic assay (S-2251) to measure residual activity (24). Using this system, plasma samples obtained from animals after high-dose plasmin infusions (e.g. 10 mg/kg body weight), contain “free” plasmin, resulting in apparent (spurious) negative α₂-antiplasmin values.

PBT and blood assay results were grouped according to coded samples and analysed without knowledge of infusate. Data were analysed using repeated measures ANOVA for overall mean differences between treatment groups, and post-hoc t-test analyses at individual time points when significant overall differences were observed. Unpaired t-tests were used to compare mean factor VIII, fibrinogen, and α₂-antiplasmin values obtained with different

Figure 3: Plasma factor VIII, α₂-antiplasmin and fibrinogen during and after 60-min infusions of escalating dosages of Δ (K2-K5) plasmin. Pre-infusion levels of α₂-antiplasmin, factor VIII, and fibrinogen were similar in all treatment groups, with means of 100 ± 6.6%, 326 ± 96%, and 292 ± 62 mg/dl, respectively. Results are calculated as % initial (pretreatment) values for each cohort of five animals receiving 1.8 (squares), 2.7 (diamonds), 3.7 (triangles), and 4.6 (circles) mg/kg of Δ (K2-K5) plasmin. Repeated measures ANOVA showed significant differences for all four concentrations of Δ (K2-K5) plasmin, with the exception of the factor VIII comparison at 1.8 vs. 2.7 mg/kg.
Results

In vitro comparison of full-length plasmin with Δ (K2-K5) plasmin for fibrin lysis efficacy

Full-length and Δ (K2-K5) plasmin induced the same dose-dependent lysis of retracted rabbit blood clots (Fig. 1). There was no difference in the dose required to initiate fibrinolysis or for maximal fibrinolytic effect at the highest dose, indicating that in vitro fibrinolytic potency of the two plasmin species is similar.

Effect of increasing dosage of Δ (K2-K5) plasmin on PBT and plasma concentrations of α2-antiplasmin, factor VIII and fibrinogen

At the lowest dose of Δ (K2-K5) plasmin (1.8 mg/kg), none of 40 PBTs was prolonged and the mean PBT during and after the start of infusion was 2.2 ± 0.7 min, not different than the pre-treatment value of 2.3 ± 0.8 min (Fig. 2, Table 1). At 2.7 and 3.7 mg/kg, significantly more PBT sites were prolonged (38% and 30%, respectively), and more of the PBT sites (70%) were prolonged using the purposely high dose of 4.6 mg/kg. Mean PBTs of 5.0 ± 2.9 min and 4.4 ± 2.2 min occurred with 2.7 and 3.7 mg/kg of Δ (K2-K5) plasmin, slightly longer than that with 1.8 mg/kg (p=0.0001 by repeated measures ANOVA). At 4.6 mg/kg, the mean PBT was longer (12.8 ± 18.8 min) than with all lower doses (p=0.001, 0.015, and 0.005 for 1.8, 2.7, and 3.7 mg/kg, respectively). Post-hoc t-test analysis at the 150-min point alone showed statistical significance (p<0.05) for these dosage comparisons, except for 2.7 vs. 4.6 mg/kg. There was no significant difference in PBT comparing 2.7 with 3.7 mg/kg.

Plasma α2-antiplasmin, factor VIII and fibrinogen concentrations decreased to nadir values at the end of the 60-min infusions (Fig. 3), with mean values showing a dose-related effect. Factor VIII and fibrinogen were completely depleted using 4.6 mg/kg Δ (K2-K5) plasmin (Fig. 4, middle and bottom), but residual amounts of factor VIII and fibrinogen (10%-35% and 30%-60%, respectively) circulated in animals receiving 1.8, 2.7 and 3.7 mg/kg. The α2-antiplasmin concentration at 30 min (Fig. 4, top) was inversely correlated with Δ (K2-K5) plasmin dose (R²=0.98), and was predictive of subsequent (60 min) levels of factor VIII (R²=0.97) and fibrinogen (R²=0.94).

Comparison of molar equivalent dosages of Δ (K2-K5) plasmin and full-length plasmin

There were more prolonged PBTs (18% vs. 0%, Chi²=6.6; p=0.01) and a longer mean PBT (3.3 ± 2.1 min vs. 2.2 ± 0.7 min, p=0.005 by repeated measures ANOVA) with 4 mg/kg full-length plasmin than with 1.8 mg/kg Δ (K2-K5) plasmin (Fig. 5; compare Tables 1 and 2). Post-hoc analysis of mean PBT at each time point after start of
infusion showed a trend for longer mean PBT with full-length plasmin at 70 min (p=0.051) and a significantly longer PBT at 150 min (p=0.019).

At higher dosages (4.6 mg/kg Δ(K2-K5) plasmin vs. 10 mg/kg full-length plasmin), the incidence of prolonged PBTs was not different (70% vs. 82% of sites, p=0.64), and the mean PBTs were likewise similar (20.9 min vs. 12.8 min, p=0.111 by repeated measures ANOVA).

Figure 6 shows the comparative plasma concentrations of α2-antiplasmin, factor VIII, and fibrinogen after exposure to these same amounts of Δ(K2-K5) plasmin or full-length plasmin. Higher dosages of both agents showed more rapid and severe depletions than did lower dosages (p<0.05), equally so with both agents (p=0.83, 0.58, and 0.76, respectively). However, at the lower dosage (1.8 mg/kg Δ(K2-K5) plasmin vs. 4 mg/kg full-length plasmin), full-length plasmin depleted plasma fibrinogen to a lower nadir level (22.2 ± 20.6% vs. 63.6 ± 9.5%, p=0.004) (bottom panel); a similar (non-significant) effect was noted for factor VIII (19.8 ± 16.4% vs. 35.2 ± 15.8%, p=0.17) (middle panel).

Discussion

Our results indicate that a novel recombinant plasmin, Δ(K2–K5), exhibits slightly better haemostatic safety in vivo in comparison with full-length human plasmin.

Escalating doses of Δ(K2–K5) plasmin exhibit a dose–response relationship on plasma concentrations of α2-antiplasmin, factor VIII, and fibrinogen (Figs. 2 and 3), in the same manner as we have shown for full-length plasmin (9). Levels of each plasma component decreased progressively during the 60-min infusions, more rapidly and to greater degree with higher dosages (Fig. 3), and with nadir values in proportion to the administered dose of Δ(K2–K5) plasmin (Fig. 4). Of importance, the mean PBT was unchanged from pre-treatment values (2.2 min vs. 2.3 min) using 1.8 mg/kg of Δ(K2–K5) plasmin, corresponding to a full-length plasmin dose (4 mg/kg) that is two-fold more than needed to lyse experimental thrombi (8). At even higher dosages of Δ(K2–K5) plasmin (2.7 and 3.7 mg/kg), the mean PBT was only slightly prolonged (5.0 and 4.4 min) (Table 1), and few sites showed prolongation beyond 4.7 min (Fig. 2). Nadir plasma factor VIII and fibrinogen at the end of infusion showed about 10–30% residual factor VIII activity and...
Table 2: Primary bleeding times (PBT) with two doses of full-length plasmin.

<table>
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<tr>
<th>Agent</th>
<th>#</th>
<th>Prolonged PBTs # (%)</th>
<th>Mean PBT (min ± SD)</th>
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<tbody>
<tr>
<td>Full-length plasmin</td>
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<tr>
<td>4 mg/kg</td>
<td>39</td>
<td>7 (18%)</td>
<td>3.3 ± 2.1</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>39</td>
<td>32 (82%)*</td>
<td>20.9 ± 26.6*</td>
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*Statistically significant differences between 4 and 10 mg/kg dosages (see text).

30–40% initial fibrinogen concentration (Fig. 4), sufficient to sustain reasonable haemostasis after traumatic injury. These data are also in agreement with prior results using full-length plasmin, showing that \( \Delta \) (K2-K5) plasmin allows intact haemostasis at thrombolytic dosages, so long as sufficient Factor VIII and fibrinogen are present in blood.

To directly test whether \( \Delta \) (K2-K5) plasmin has similar effects on haemostasis as full-length plasmin, we compared molar equivalent doses of the agents in a blinded manner. At the purposefully toxic dosages of 4.6 mg/kg of \( \Delta \) (K2-K5) plasmin and 10 mg/kg of...
**What is known about this topic?**
- “Delta” plasmin is a newly-described recombinant derivative of plasmin that lacks kringles 2 to 5.
- Full-length plasmin is under current clinical trial in patients with peripheral arterial graft occlusion and acute ischaemic stroke.

**What does this paper add?**
- Delta plasmin has equal fibrinolytic effect in vitro as full-length plasmin.
- Delta plasmin exhibits a better haemostatic safety profile than full-length plasmin in an animal model of induced bleeding.

full-length plasmin, significant prolongation of PBT occurred equally with both agents, to 12.8 ± 18.8 min vs. 20.9 ± 26.6 min, p=0.12 (Tables 1 and 2, Fig. 5). Further, both agents induced equally rapid and complete depletion of plasma α2-antiplasmin, factor VIII, and fibrinogen (Fig. 6). At a lower dosage (1.8 mg/kg Δ (K2-K5) plasmin vs. 4 mg/kg full-length plasmin), Δ (K2-K5) plasmin showed slightly fewer prolonged PBTs (0% vs. 18%, Chi²=6.6; p=0.01), and a slightly shorter mean PBT (2.2 ± 0.7 vs. 3.3 ± 2.1 min, p<0.001 by repeated measures ANOVA). Although there were only five observations at individual time points after each infusion, post-hoc t-test data were significant at 150 min (p=0.019) and strongly suggestive at 70 min (p=0.051) of less bleeding with Δ (K2-K5) plasmin. The observed better haemostatic safety for Δ (K2-K5) plasmin may be explained by the higher residual plasma fibrinogen concentrations in animals treated with Δ (K2-K5) plasmin (Fig. 6 bottom), providing more cloggable substrate to participate in clot formation after the traumatic ear punctures.

It is possible that the observed greater haemostatic safety of Δ (K2-K5) plasmin over full-length plasmin reflects lower fibrinolytic potency, a more rapid plasma clearance or an unexplained lower in vivo thrombolytic potential. Our comparison of Δ (K2-K5) plasmin with full-length plasmin for in vitro clot lysis (Fig. 1) shows that equimolar amounts of each agent induce the same degree of clot lysis, indicating that Δ (K2-K5) plasmin possesses equivalent binding and catalytic properties. On this basis, the data suggest that Δ (K2-K5) plasmin may possess greater haemostatic safety, but further study is needed to determine whether clinical efficacy is equal to that for full-length plasmin.

**Disclosures**
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**References**

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