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REGULAR ARTICLE

The thrombolytic effect of miniplasmin in a canine model of femoral artery thrombosis

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Abstract

Background and purpose: Miniplasmin was a des-kringle variant of plasminogen with potential pharmacological application. We investigated the thrombolytic effect of miniplasmin in a canine model of femoral artery thrombosis.
Methods: In anesthetized dogs, a stable occlusive thrombus was formed by mechanical and electrolytic injury of the vessel wall, that the animals were later injected with miniplasmin (0.75 mg/kg, 1.5 mg/kg and 3.0 mg/kg, i.a.) and rt-PA (0.5 mg/kg, i.a.) intra-arterially. Hemodynamic parameters and hemorrhage status were monitored for 2 h. Thrombin time, activated partial thromboplastin time, prothrombin time and fibrinogen concentration were tested at 2 h after administration. Fibrin degradation product and D-dimer concentration were tested by ELISA.
Results: The incidence of reperfusion in the miniplasmin (3.0 and 1.5 mg/kg) groups was 100%, and time to reperfusion was (3.3 ± 1.0) and (7.0 ± 2.3) min, which was shorter than rt-PA. After reperfusion, none of the vessels in the miniplasmin (1.5 and 3.0 mg/kg) groups reoccluded, whereas 20% of vessels reoccluded in the rt-PA group. Rudimental thrombus mass in the miniplasmin (1.5 and 3.0 mg/kg) groups were smaller than rt-PA. The operative wounds in all miniplasmin groups had no hemorrhage within 2 h. There were no significant differences in thrombin time, activated partial thromboplastin time and prothrombin time. Fibrinogen concentration in the miniplasmin (3.0 mg/kg) group reduced significantly as compared with baseline and thrombosis values, whereas these values in the miniplasmin (1.5 and 0.75 mg/kg) groups were unchanged. Fibrin degradation product and D-dimer concentration increased significantly after thrombolysis.
Conclusions: The results suggest that miniplasmin may be useful for the treatment of thrombosis and without complication of hemorrhage. This is in contrast to rt-PA, which intrinsically has a higher risk of occurring the hemorrhage risk.

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Introduction

Therapeutic thrombolysis with plasminogen activators is an effective and potentially life-saving measure in the management of patients with acute myocardial infarction [1,2] and peripheral arterial or graft occlusion (PAO) [3]. However, every thrombolytic agent thus far utilized carries a tangible risk of life-threatening hemorrhage. While direct administration of the agent into a venous or arterial thrombus induces more efficient thrombolysis, bleeding at remote sites cannot be entirely avoided, probably because the activator is distributed systemically during and after vascular reperfusion. Thus, the incidence of intra-cranial hemorrhage (ICH) is 0.4–2.0% of patients with acute myocardial infarction, deep venous thrombosis or pulmonary embolism treated intravenously [4–6] and ICH occurs in 1–2% of patients with PAO who receive local infusions for up to 2 days [3,7], especially with concomitant heparin administration [8].

Agents that barely affect the plasma fibrinogen concentration can still cause bleeding [9], reinforcing the concept that fibrinolytic hemorrhage is the result of the haemostatic plug dissolution at vascular injury sites. Currently, no thrombolytic agent is demonstrated to have clinical efficacy without bleeding risk.

Plasminogen is easily obtained from human plasma or plasma fractions. Its activation is affected by digestion of the peptide bond between arginine 561 and valine 562 (R^{561} V^{562}) by tissue plasminogen activator (tPA) or urokinase (uPA) trapped in the blood clot. Plasminogen cannot readily be expressed in eukaryotic expression systems but has been obtained from the baculovirus cell system, which is however, not suitable for large-scale production. During the early investigations of thrombolytic approaches, plasmin was recognized as a direct fibrinolytic enzyme with potential clinical application. In the context of local administration, the theoretical limitations of plasmin, namely, inactivation by antiplasmin in the blood might actually provide a mechanism for safety from hemorrhage. Plasmin is unstable in neutral solutions, but can be stabilized by specific amino acids (such as lysine or tranexamic acid), acid pH (range 2–4) or glycerol (10–50%). Plasmin contains two chains. The chain A of the plasmin molecule consists of five triple-loop disulfide kringle (Kr) domains, while the chain B contains a "linker" region of 20 amino acids and a serine protease domain. Miniplasmin [10] in the study consists of only the kringle-5 domain, the linker, and the serine protease domain, which is different from recombinant microplasmin (μPlm) [11] that consists of only the linker and serine protease domain. The inhibition of miniplasmin and μPlm by α_2-antiplasmin (AP) occurs slowly when compared with plasmin, reflecting the absence of the non-covalent binding site of K1-3 [11,12]. The inhibition of miniplasmin is slower than μPlm due to the kringle-5 domain, which can slow down the action by increasing stereospecific blockade. MiniPlasminogen is refolded in *Escherichia coli* inclusion bodies and activated into miniplasmin by urokinase (uPA). Miniplasmin is stabilized in a dilute citrate buffer at pH 3–4. Miniplasmin in the study is prepared by PTI Company (USA), and spatial configuration modification is performed by Fuchun-zhongnan Company (China).

The present study evaluates the thrombolytic effect of miniplasmin in a canine model of femoral artery thrombus and the hemorrhage risk of miniplasmin as compared with rt-PA.

Materials and methods

Drugs and reagents

Miniplasmin was from Shanghai Fuchun-zhongnan Biotech Company (China). Recombinant tissue-type plasminogen activator (rt-PA; actilyse) was from Boehringer Ingelheim (Germany). All other reagents used in this study were obtained from commercial sources.

Animals

Experiments were performed on adult male Beagle dogs weighing 9–12 kg, which were obtained from Academy of Military Medicine Sciences (Beijing, China). At all times, the animals received care in compliance with the criteria in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences. Animal procedures performed were in accordance to the guidelines of the Chinese Society of Laboratory Animal Sciences.

Surgical procedure

Dogs were anesthetized with 30 mg/kg intravenous sodium pentobarbital. For replacement of fluid loss, 10 mL/kg/h saline was given continuously throughout the experiment. Oesophageal temperature was monitored and maintained at 37±0.5 °C with an electric blanket and a heat lamp. The femoral thrombosis model used in this study was performed by injuring vascular wall [13,14]. The left femoral artery and femoral vein were dissected, a 3–4 cm section of each vessel and a side-branch of the femoral artery were carefully exposed. The side-branch was isolated for administration of drugs and contrast medium. The femoral artery was instrumented with a 3.0-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA) for continuous measurement of blood flow. The endothelium of the femoral artery was injured by gently squeezing with a hemostat clamp. After perfunctory injury, the Goldblatt clamp on the femoral artery was adjusted to reduce flow by approximately 80%, and a 150-μA anodal current electrode was placed around the femoral artery for 30 min due to thrombus formation. The sides of injured blood vessel were clipped by two bulldog clamps for 50–60 min, and then thrombus was performed if the flow was reduced to 0 mL/min.