Vampire Bat Plasminogen Activator DSPA-Alpha-1 (Desmoteplase): A Thrombolytic Drug Optimized by Natural Selection

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Key Words

Plasminogen activator · DSPA ·
Desmoteplase · Thrombolytic · Fibrinolytic ·
Fibrin specificity · Stroke

Abstract

Plasminogen activators are enzymes found in all vertebrate species investigated so far. Their physiological function is the generation of localized proteolysis in the context of tissue remodeling, wound healing and neuronal plasticity. The common vampire bat (*Desmodus rotundus*) is a New World species that feeds exclusively on blood. Its saliva contains highly potent plasminogen activators, specialized in rapid lysis of fresh blood clots. Biochemical and pharmacological evidence indicates that these plasminogen activators represent a new class of thrombolytics with pharmacological and toxicological proper-

ties superior to human tissue-type plasminogen activator, the clot dissolving agent now most frequently used in medicine. A form of the enzyme produced by recombinant DNA technology is currently employed to test this hypothesis in clinical studies.

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Introduction

Thrombolytic therapy with plasminogen activators (PAs) is performed routinely as a treatment of acute myocardial infarction as well as selected cases of cerebral vascular occlusion and pulmonary embolism. Thrombolytic agents such as streptokinase (SK), tissue-type PA (t-PA), and urokinase-type plasminogen activators (u-PA) are potent drugs, but they have various shortcomings related to their pharmacodynamic, pharmacokinetic

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and safety profiles. Vampire bats are the only mammalian species feeding exclusively on blood. To support this diet, their saliva contains very potent PAs, originally described by Hawkey [1]. In contrast to t-PA with its more subtle role in wound healing and neuronal plasticity, vampire bat salivary PA has been optimized by natural selection for the rapid lysis of fresh blood clots.

Biochemistry

In order to exploit this extraordinary evolutionary adaptation for medical purposes, we have cloned, expressed and characterized salivary PAs from the vampire bat *Desmodus rotundus*. Four *D. rotundus* salivary PAs (DSPAs) were identified which, like t-PA and u-PA, are composed of various conserved domains known from related proteins. DSPA α_1 and DSPA α_2 exhibit the structural formulas finger (F), EGF (E), kringle (K), protease (P), and DSPA β and DSPA γ , the formulas EKP and KP, respectively. Subtle sequence differences and data from Southern blot hybridization indicate that the four enzymes are coded by four different genes and are not generated by differential splicing of a single primary transcript [2, 3]. A preliminary biochemical and pharmacological analysis indicated that DSPA α_1 exhibited the most favorable profile and was therefore chosen for further study. A recombinant CHO cell line for the production of DSPA and a purification protocol were established to obtain material that fulfilled the specifications for preclinical and clinical development [4]. The most important feature that distinguishes DSPA from other plasminogen activators is its extraordinary fibrin specificity. In fact, the activity of DSPA α_1 is 105,000 times higher in the presence of fibrin than in its absence. The respective factor for t-PA is only 550. Likewise, fibrinogen, a fairly

potent cofactor of plasminogen activation by t-PA, has hardly any effect on DSPA α_1 . Therefore the factor of fibrin selectivity expressed as the quotient of activity in the presence of fibrin versus activity in the presence of fibrinogen is 12,900 for DSPA but only 72 for t-PA [5].

In order to explore the molecular basis of this fastidious dependence on a fibrin cofactor, the recombinant catalytic domain of DSPA α_1 has been crystallized in a covalent complex with Glu-Gly-Arg-chloromethyl ketone and its structure solved at 2.9 Å resolution. The structure is similar to that of activated two-chain human t-PA. Despite its single-chain status, the activation domain is observed in an enzymatically active conformation, with a functional substrate binding site and an active site accommodating the peptidylmethylene inhibitor. The activation pocket, which normally receives the N-terminal Ile16, is occupied by the side chain of Lys156, whose distal ammonium group forms an internal salt bridge with the carboxylate group of Asp194. Lys156 is in a groove shielded from the bulk solvent by the intact 'activation loop' (Gln10-Phe21), favoring Lys156-Asp194 salt bridge formation and stabilization of a functional substrate binding site. Together with the characteristic 186 insertion loop, the activation loop could act as a switch, effecting full single-chain enzymatic activity upon binding to fibrin [6].

Neurotoxic Potential of t-PA

Kingston et al. [7] studied the effects of Alzheimer amyloid β -peptide analogues on the activity of t-PA in vitro. These authors discovered that the peptides exhibited a marked stimulatory effect upon plasminogen activation by t-PA, similar to known stimulators of t-PA such as fibrinogen. This activity

appeared to increase when β -peptides formed aggregated fibrillar structures equivalent to those found in amyloid deposits. This finding may have a bearing on the pathogenesis of hereditary cerebral hemorrhage with amyloidosis Dutch type and cerebral amyloid angiopathy-related cerebral hemorrhage. Such a mechanism may contribute to the intracerebral hemorrhages that have occurred in patients undergoing t-PA treatment for acute myocardial infarction. In contrast to t-PA, DSPA α_1 is neither stimulated by native nor by aggregated amyloid β -peptide analogues [Bringmann P., Donner P. and Schleuning W.-D., unpubl. obs.]. Besides its desired thrombolytic effects, t-PA also promotes neurodegeneration after intracerebral injection of excitotoxins, such as glutamate, and contributes to the spread of neuronal damage after a cerebral vascular occlusion, which is believed to be promoted by excitotoxins. Wang et al. [8] examined the role of t-PA in experimental cerebral ischemia, independent of its effect as a thrombolytic agent. t-PA-deficient mice exhibited approximately 50% smaller cerebral infarcts than wild-type mice. Intravenous injection of t-PA into t-PA $^{-/-}$ or wild-type mice produced larger infarcts, indicating that t-PA increased stroke-induced injury. Since t-PA promotes desirable (thrombolytic) as well as undesirable (neurotoxic) manifestations during stroke, a thrombolytic agent that does not promote excitotoxic damage could achieve better neuroprotection after an acute cerebral infarct.

Pharmacology

On the basis of biochemical evidence, DSPA α_1 fulfills the hopes and expectations originally placed on t-PA: plasminogen activation restricted to the clot surface, without the systemic activation that leads to fibrino-

gen consumption, 'plasminogen steal', and degradation of clotting factors VIII and V. Pharmacological studies using a lung embolism model in rats demonstrated that recombinant DSPA α_1 is more potent and more clot specific than t-PA [9]. A higher potency, clot specificity, and prolonged half-life of DSPA α_1 over t-PA were also verified in an arterial thrombosis model in rats [10]. In a coronary thrombosis model of acute myocardial infarction in dogs, DSPA α_1 led to a faster recanalization and a lower incidence of reocclusion compared to t-PA [11]. On the other hand, a lower incidence of bleeding was observed with DSPA α_1 in a rat mesenteric vein model [12]. DSPA α_1 was characterized pharmacokinetically in rats and cynomolgus monkeys. In comparison to t-PA, a lower total clearance and a longer terminal half-life could be demonstrated [13]. These data encouraged the development of an intravenous bolus regimen in humans. A detailed toxicologic study has been performed in rats and monkeys. No direct toxic effects could be observed, and secondary effects appeared only at extremely high doses of DSPA α_1 .

Clinical Studies

Myocardial Infarction

A phase II study was conducted to evaluate the efficacy, safety and tolerability of DSPA α_1 as a thrombolytic agent in the treatment of patients with acute myocardial infarction. The study was designed as a non-randomized, open-label, prospective dose finding study in which patients received an intravenous bolus of either 0.5 mg/kg or 0.75 mg/kg of DSPA α_1 administered over 1–2 min followed by intravenous heparin. Efficacy was determined by measuring coronary angiography patency rates at 90 min after onset of thrombolysis while safety was as-

sessed by recording the occurrence of bleedings, allergic and other early or late complications. In addition, the impact of DSPA α_1 on hemostatic parameters and the development of DSPA α_1 antibodies after treatment were determined. A total of 26 patients (19 males and 7 females) were enrolled into the study with a mean age of 61 years (range 41–75) and a mean weight of 76 kg (range 62–92). The follow-up period of observation was 6 months. 18 patients received 0.5 mg/kg and 8 patients received 0.75 mg/kg of DSPA α_1 . Patency, as defined by a TIMI grade III score at the 90-min coronary angiogram, was achieved in 65% (17 of the 26 patients). Late patency (90 min–24 h) was demonstrated in 21 patients. Out of the remaining 5 patients 3 died within 8 h after inclusion and 2 refused the 24- to 36-hour angiogram. The laboratory data from the study confirmed the high fibrin specificity of DSPA α_1 demonstrated by normal levels of fibrinogen. Other laboratory parameters – plasminogen, hematocrit, hemoglobin, activated partial thromboplastin time, creatine kinase, creatine kinase-MB and α_2 -antiplasmin – did not indicate any treatment-related safety issues. The safety profile of DSPA α_1 in this early study was typical for the administration of a thrombolytic in myocardial infarction. The serious adverse event reported was judged not to be DSPA α_1 -related by the independent safety advisory board. These events were rather indication specific and typical for the natural course of the disease. This early study demonstrated that DSPA α_1 acted as a typical plasminogen activator with comparable activity to other thrombolytics: The patency rate was 65%. Fibrin specificity was demonstrated by the fact that no fibrinogen depletion occurred. Practically none of the bleeding episodes required any therapy. Bleeding occurred more frequently in studies where angiography was performed and when patients were treated

with high doses of heparin. Conclusions with regard to efficacy and safety are hampered by the fact that the patient number was small; however, proof of concept (thrombolysis) could be shown [G. Groetzbach, D. Gulba et al., pers. commun.].

Stroke

After the favorable pharmacotoxicological and pharmacokinetic profile of DSPA α_1 had been confirmed by the results of the phase II study in acute myocardial infarction, a phase II/III study of desmoteplase in the indication acute cerebral vascular occlusion appeared promising. The Desmoteplase in Acute Ischemic Stroke study was initiated by Paion GmbH, Stolberg, Germany in January 2001. It is an international multicenter placebo-controlled study in the time window 3–6 h after onset of stroke using MRI-measured mismatch as a key inclusion criterion next to standard inclusion and exclusion criteria typical for the use of a thrombolytic. Centers have to be qualified by a central imaging unit for their MRI capabilities. First results of the study will become available in 2002.

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