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Desmoteplase

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Thrombolytic Plasminogen Activator

rDSPAα_1
SH-576

Recombinant protein corresponding to a natural plasminogen activator (isoform α_1) from the saliva of the vampire bat *Desmodus rotundus*

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Abstract

Desmoteplase is a plasminogen activator in phase III trials at PAION, Forest and Lundbeck for the treatment of acute ischemic stroke. PAION is also developing the drug candidate for the treatment of pulmonary embolism. Desmoteplase is a genetically engineered version of a clot-dissolving protein found in the saliva of the vampire bat *Desmodus rotundus*. It possesses high fibrin selectivity, allowing it to dissolve a clot locally without affecting the blood coagulation system, which is thought to potentially reduce the risk of intracranial bleeding as compared to less fibrin-specific plasminogen activators. In the U.S., desmoteplase has received fast track designation for the treatment of ischemic stroke beyond the 3-h time window.

Preparation and Biochemistry

Salivary plasminogen activators (PAs) from the vampire bat *Desmodus rotundus* have been cloned, expressed and characterized (1). Four *D. rotundus* salivary PAs (DSPAs) were identified and, like tissue-type PA (t-PA) and urokinase-type PA (u-PA), are composed of various conserved domains known from related proteins. In the t-PA molecule there are several regions responsible for high-affinity binding to fibrin (fibronectin finger region, F), for binding to receptors in the liver (epidermal growth factor [EGF] and kringle-1), and for low-affinity binding to fibrin (kringle-2 region, K). The protease (P) domain is responsible for the cleavage of plasminogen and also binds plasminogen activator inhibitor type-1 (PAI-1), inhibiting the proteolytic function of t-PA.

DSPAα_1 and DSPAα_2 contain F, EGF (E), K and P domains, and DSPAβ and DSPAγ contain EKP and KP domains, respectively. The four enzymes are coded by four different genes and are not generated by differential splicing of a single primary transcript (2, 3). Biochemical and pharmacological analysis indicated that DSPAα_1 exhibited the most favorable profile. The most important feature that distinguishes DSPA from other PAs is its extraordinary fibrin specificity. In fact, the activity of DSPAα_1 is 105,000 times higher (only 550 for t-PA) in the presence of fibrin than in its absence (4).

To investigate the molecular basis of the fibrin dependency, the recombinant catalytic domain of DSPAα_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, the distal ammonium group of which 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 (5).

Recombinant DSPAα_1 is produced in transformed Chinese hamster ovary (CHO) cells (6, 7). In order to improve the production system to provide sufficient amounts of the substance at low cost, different alternatives, such as transgenic tobacco plants and suspension plant cells (BY-2), have been tested. However, although production was demonstrated to be feasible in both models and product accumulation was significant, production was hampered by proteolysis (8).

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Background

Fibrinolysis is in essence the dissolution of consolidated thrombi. The central reaction of the fibrinolytic system is conversion of the inactive proenzyme plasminogen to the proteolytic enzyme plasmin through cleavage of a single peptide bond by specialized PA proteases. 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 generated plasmin digests fibrin to soluble degradation products. Conversion of plasminogen to plasmin is activated intravascularly through the intrinsic pathway by factor XIIa, the initiator of the coagulation cascade, and extravascularly through the extrinsic pathway by "tissue activators".

Thrombolytic therapy with PAs is performed routinely in the treatment of acute myocardial infarction (AMI) (9), as well as selected cases of cerebrovascular occlusion (10) and pulmonary embolism (PE) (11, 12). Thrombolytic agents such as streptokinase, t-PA and u-PA are potent drugs, but they have various shortcomings related to their pharmacodynamic, pharmacokinetic and safety profiles. Fibrinolytic agents should exhibit fibrin specificity to avoid extensive systemic plasminogen activation and degradation of other plasma proteins, which may cause greater systemic coagulopathy, with an increased risk of bleeding (13). Streptokinase and urokinase are first-generation fibrinolytic agents demonstrated to be effective in inducing thrombolysis. However, these drugs exhibit low fibrin specificity, and are able to convert circulating plasminogen to plasmin. In addition, streptokinase may induce immunogenic reactions. t-PA and single-chain urokinase-type plasminogen activator (scu-PA, also called prourokinase) are second-generation agents. Both activators bind avidly to fibrin, enabling them to cause efficient and localized digestion of the clot or thrombus. As derived from results of clinical trials, the high doses of these agents required produce a slight to moderate decrease in levels of fibrinogen and plasminogen, indicating that the fibrin selectivity is rather limited and is influenced by the dose and duration of the infusion (14, 15). Third-generation PAs have been developed in order to lengthen the half-life of the drug, increase resistance to plasma protease inhibitors, improve the specificity for fibrin (16) and diminish the immunogenicity. In addition, highly potent PAs specialized in rapid lysis of fresh blood clots have been found in the saliva of vampire bats.

The common vampire bat *D. rotundus* is a New World species that feeds exclusively on blood. To support this diet, their saliva contains very potent PAs, originally described by Hawkey (17). 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. Biochemical and pharmacological evidence indicates that these PAs represent a new class of thrombolytics with pharmacological and toxicological properties superior to human t-PA, the clot-dissolving agent now most frequently used in medicine. Desmoteplase (rDSPAα_1), a form of the enzyme produced by recombinant DNA technology, possesses high fibrin selectivity, potentially allowing it to dissolve a clot locally without adversely affecting the blood coagulation system, possibly reducing the risk of intracranial bleeding as compared to other less fibrin-specific PAs.

Preclinical Pharmacology

Based on the biochemical evidence, DSPAα_1 was seen as a promising thrombolytic agent since plasminogen activation is restricted to the clot surface, without the systemic activation that leads to fibrinogen consumption, 'plasminogen steal' and degradation of clotting factors VIII and V. Pharmacological studies using both lung embolism and arterial thrombosis models in rats and rabbits demonstrated a higher potency and clot specificity and a prolonged half-life for recombinant DSPAα_1 compared to t-PA (18, 19). In a coronary thrombosis model of AMI in dogs, DSPAα_1 led to a faster re-canalization and a lower incidence of re-occlusion compared to t-PA (20). On the other hand, a lower incidence of bleeding was observed with DSPAα_1 in a rat mesenteric vein model (21).

Experiments with DSPAα_1 fused to an antibody to P-selectin have been performed in animal models (22) in order to improve local thrombolysis and reduce bleeding time, based on the knowledge that P-selectin is expressed on the surface of activated endothelial cells and platelets during thrombosis. When compared to DSPAα_1, the fusion protein had similar thrombolytic efficacy in a rat PE model and antithrombotic potency in a dog model of femoral artery thrombosis. However, it was less effective in inducing lysis of pre-existing arterial thrombi in the dog model, probably due to the lack of stimulation by fibrin in arterial thrombi and not to the pharmacokinetic properties of the fusion protein.

Pharmacokinetics

Pharmacokinetic characterization of DSPAα_1 in rats and cynomolgus monkeys, performed using a specially developed ELISA method (23), demonstrated a lower total clearance and a longer terminal half-life in comparison to t-PA (24). Sex-dependent differences were not observed. By means of allometric extrapolation, a total clearance of approximately 1 ml/min/kg was predicted for humans (24). These data encouraged the development of an i.v. bolus regimen in humans.

In healthy volunteers, after single bolus injections of 0.01 to 0.05 mg, DSPAα_1 was eliminated following a biphasic elimination curve with a $t_{1/2\alpha}$ of only 4 min and a $t_{1/2\beta}$ of 2.3-2.7 h, the second elimination phase being responsible for the clearance of 83% of the substance (25). At these doses, no effect on endogenous coagulant and fibrinolytic parameters was detected. In addition, the substance appeared to be completely fibrin-selective

and did not cause any procoagulant effect (25). Thus, unlike rt-PA, with a half life of only 11-14 min (26), or staphylokinase, with a half-life of 6.3 min (27), results confirm that DSPAα_1 is suitable for administration as a single bolus injection. The slow clearance of DSPAα_1 may help to prevent early re-occlusion after successful thrombolysis, contributing to better post-infarct survival.

Safety

Apart from its well-established fibrinolytic role, t-PA acts as an effector within the central nervous system, where it is produced by endothelial cells (28). t-PA is also expressed by neuronal and microglial cells and contributes to neuronal plasticity and long-term memory formation (29). However, in addition to its neurophysiological roles, t-PA promotes excitotoxic (30) and ischemic injury within the brain. These findings have implications for the use of t-PA in the treatment of acute ischemic stroke. In studies performed in mice, the ability of t-PA and DSPAα_1 to promote kainate- and NMDA-induced neurodegeneration was evaluated (31, 32). It was demonstrated that, in contrast to t-PA, DSPAα_1 does not promote excitotoxic injury either when injected directly into the brain (31) or when administered intravenously (32). In addition, DSPAα_1 showed an antagonistic effect on t-PA potentiation of NMDA-mediated neurotoxicity, probably due to the fact that both molecules share structural characteristics (32). The authors concluded that while DSPAα_1 has evolved to specifically promote fibrin dissolution to support feeding, t-PA appears to be a protease with pleiotropic functions associated with structural elements other than the classical active site. One of the most striking sites is the lysine-binding kringle II domain of t-PA, absent in DSPAα_1 (32).

It should also be mentioned that, in contrast to t-PA, DSPAα_1 is not stimulated either by native or aggregated β-amyloid peptide analogues (from Ref. 1).

As a nonhuman protein, there are concerns that rDSPAα_1 may have potential for severe antigenicity, not only by inducing the formation of neutralizing antibodies but also by provoking allergies. The potential for antigenicity was tested in rats and monkeys. After a single bolus injection of 3 or 10 mg/kg, none of the rats developed antibodies against DSPAα_1 (33). Even after repeated i.v. administration (4 times 7 days apart) of 1, 3 or 10 mg/kg, inconsistent low-titer antibody formation was observed. The formation of antibodies was more frequently observed after repeated administration but was only uniformly observed in monkeys when two doses of 30 mg/kg were injected 9 days apart (25). In monkeys, which after transient expression had nondetectable DSPAα_1 antibody levels, immunological memory was observed, causing booster reactions upon a second exposition that remained for up to 1 year. However, findings did not indicate any allergic reaction caused by DSPAα_1 (25). In studies performed in healthy human volunteers, no signs of production of IgG or IgM antibodies to DSPAα_1 were detected in any of the subjects (25).

The fibrinolytic activity of DSPAα_1 was not affected in any of the animals that developed antibodies to the molecule (33). In addition, considering the molecular similarity between DSPAα_1 and t-PA, no cross-reactivity of the DSPAα_1 antibodies with rt-PA was observed (25).

Clinical Studies

A phase II study designed as a prospective, nonrandomized, open-label, dose-finding trial was conducted to evaluate the efficacy, safety and tolerability of DSPAα_1 as a thrombolytic agent in the treatment of patients with AMI. Patients received an i.v. bolus of either 0.5 or 0.75 mg/kg of DSPAα_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. Safety was assessed by recording the occurrence of bleeding, allergic and other early or late complications, and the formation of antibodies. The effect of DSPAα_1 on hemostatic parameters was also determined. Twenty-six patients (19 males and 7 females) with a mean age of 61 years (range: 41-75) and a mean weight of 76 kg (range: 62-92) were enrolled. The follow-up period of observation was 6 months. Eighteen patients received 0.5 mg/kg and 8 patients received 0.75 mg/kg of DSPAα_1. Patency, as defined by a TIMI grade III score at the 90-min coronary angiogram, was achieved in 65% (17 of 26 patients). Late patency (90 min-24 h) was obtained in 21 patients. Of the remaining 5 patients, 3 died within 8 h after inclusion and 2 refused the 24-36-h angiogram. The normal levels of fibrinogen confirmed the high fibrin specificity of DSPAα_1. Other laboratory parameters remained unchanged. DSPAα_1 acted as a typical PA in terms of safety profile and achieved a patency rate (65%) comparable to other thrombolytics. Bleeding episodes rarely required any therapy and occurred more frequently in studies where angiography was performed. However, the number of patients included was too small to infer definitive conclusions (from Ref. 1).

In the phase II DEPTH (DEsmoteplase in Pulmonary THromboembolism) study, an open-label, multinational, randomized, parallel-group study, three doses of desmoteplase (125, 180 and 250 μg/kg) were tested and compared to 100 mg alteplase (rt-PA) for the treatment of PE. With regard to efficacy, a dose-response was demonstrated 24 h after treatment: 180 and 250 μg/kg desmoteplase reduced mean pulmonary artery pressure (mPAP) similarly to 100 mg rt-PA. Desmoteplase showed a good safety profile, with no dose-dependent increase in major bleeding episodes and bleeding rates similar to those observed with rt-PA. No change in fibrinogen level was observed. The results of this study extended the possible indication profile for desmoteplase to PE (34).

The only drug approved for acute ischemic stroke is rt-PA, although its use is limited by the need to administer it within 3 h of symptom onset (35). After the favorable pharmacotoxicological and pharmacokinetic profile of DSPAα_1 had been confirmed by the results of the phase II study in AMI, two phase II studies of desmoteplase in

acute cerebrovascular occlusion appeared promising: the European/Australasian Desmoteplase in Acute Ischemic Stroke (DIAS), and the U.S. Dose Escalation study of Desmoteplase in Acute Ischemic Stroke (DEDAS). DIAS (36) and DEDAS (37) were multinational, double-blind, placebo-controlled, dose-finding phase II safety and efficacy studies. In both these studies, eligibility criteria included baseline National Institute of Health Stroke Scale (NIHSS) scores of 4-20 and magnetic resonance imaging (MRI) evidence of perfusion/diffusion mismatch. Patients were then administered i.v. desmoteplase or placebo in the time window up to 9 h after the onset of stroke symptoms. Efficacy endpoints were the rate of reperfusion on MRI after 4-8 h and clinical outcome as assessed by NIHSS, modified Rankin scale and Barthel Index at 90 days. Both of these studies were pilot in nature and were not sized to show statistical differences. DIAS was the first prospective, randomized, placebo-controlled acute stroke thrombolysis trial to use MRI both for patient selection and as a primary efficacy endpoint (36). In part I, DIAS began with a dose-ranging design investigating fixed doses between 25 mg (median of 313 μg/kg) and 50 mg (median of 546 μg/kg), although excessive symptomatic intracranial hemorrhage (sICH) was observed. In part II, lower weight-adjusted doses escalating through 62.5, 90 and 125 μg/kg were subsequently investigated in 57 patients. The lowest dose (62.5 μg/kg) did not cause significant reperfusion.

Considering DIAS and DEDAS, altogether 142 acute ischemic stroke patients were randomized in the U.S., Europe and Australasia within 3-9 h from the onset of stroke symptoms (38). The joint analysis included all patients treated with placebo, 90 or 125 μg/kg desmoteplase. The combined rate of intracranial bleeding for the doses of 90 and 125 μg/kg desmoteplase was 1.7% (1 patient in the lower dose group). Mortality was generally low: 5.7% on placebo, 6.9% on 90 μg/kg and 3.3% on 125 μg/kg. The reperfusion rates were 23.5% on placebo, 34.6% on 90 μg/kg and 62.1% on 125 μg/kg. Positive clinical outcomes at day 90 were achieved in 22.9% on placebo, 37.9% on 90 μg/kg and 60% on 125 μg/kg. It was concluded that there was a very low rate of sICH at the doses investigated. Both reperfusion and clinical outcome were substantially and significantly improved with the higher dose of desmoteplase compared to placebo (36-38).

DIAS-2 is an ongoing phase IIb/III trial aimed to confirm the 3-9-h treatment time window of desmoteplase in ischemic stroke in a larger number of patients. Perfusion computed tomography (pCT) and MRI are both allowed as diagnostic tools for the identification of patients who may benefit from reperfusion therapy with desmoteplase.

Conclusions

New thrombolytic agents offer pharmacokinetic and dynamic advantages over t-PA, the clot-dissolving agent now most frequently used in medicine. Desmoteplase, or DSPAα_1, is one of four plasminogen activators originally derived from the saliva of the vampire bat *D. rotundus* and now produced by recombinant DNA technology. DSPAα_1 has been optimized by natural selection for the rapid lysis of fresh blood clots. It exhibits enhanced fibrin specificity with a strict requirement of polymeric fibrin as co-factor, and therefore it is expected to have superior potency as compared to t-PA. Desmoteplase demonstrates minimal neurotoxicity, has a long half-life enabling administration as a single bolus, and its slow clearance may help to prevent early re-occlusion after successful thrombolysis. Results from phase II studies suggest that desmoteplase may be beneficial for stroke treatment in a time window of up to 9 h after the onset of symptoms. Clinical proof of concept for desmoteplase has been obtained in three indications: acute ischemic stroke, AMI and PE.

Sources

PAION AG (DE) (licensed from Schering AG); licensed to Forest Laboratories, Inc. for the U.S. and Canada and to H. Lundbeck A/S for Europe, Japan and the rest of the world.

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