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Amediplase

Prop INN

Treatment of Acute Myocardial Infarction
Thrombolytic
Tissue-Type Plasminogen Activator

CGP-42935
K_2tu-PA

173-L-Serine-174-L-tyrosine-175-L-glutamine-173-275-plasminogen activator (human tissue-type reduced), fusion protein with urokinase (human urine β-chain reduced)

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Abstract

Third-generation plasminogen activators were developed in order to lengthen the half-life of the drug, to increase resistance to plasma protease inhibitors, to improve fibrin specificity and to diminish immunogenicity. Amediplase is one such agent that in animal models has been shown to be more potent and longer-acting than alteplase, the standard thrombolytic drug for the treatment of acute myocardial infarction. Amediplase has the advantage over other thrombolytic agents that it can be administered as a single bolus. In clinical studies to date, amediplase has demonstrated a good safety profile and a low incidence of serious bleeding complications.

Description and Production

Amediplase (K_2tu-PA) is a hybrid plasminogen activator in which the kringle-2 domain of tissue-type plasminogen activator (t-PA) is linked to the catalytic protease domain of single chain urokinase-type plasminogen activator (scu-PA) (Fig. 1). K_2tu-PA was constructed by standard recombinant technology with fusion of cDNAs encoding t-PA and encoding scu-PA. The correct construction was made using oligonucleotide site-directed mutagenesis. K_2tu-PA was produced in a CHO cell line using a mouse cytomegalovirus-promoter/enhancer-driven expression cassette. The hybrid was harvested from the culture medium and purified using a combination of ion exchange chromatography, hydrophobic interaction chromatography and gel filtration. Purification was to homogeneity, as established by SDS-chromatography and silver staining (1-3).

Introduction

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 plasminogen activator (PA) proteases. 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.

Fibrinolytic therapies are indicated for the treatment of acute thrombus occlusion of coronary arteries that leads to myocardial infarction (4). At early stages, acute myocardial infarction is frequently associated with thrombotic coronary artery occlusion. One approach to treatment consists of pharmacological dissolution of the blood clot by intravenous infusion of plasminogen activators that activate the fibrinolytic system. Rapid coronary reperfusion limits infarct size, decreases ventricular dysfunction and improves survival.

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 (5). Streptokinase and urokinase are first-generation fibrinolytic agents that have been demonstrated to be effective in thrombolysis. However, these drugs exhibit low fibrin specificity and are able to convert circulating plasminogen to plasmin. In addition, streptokinase may induce immunogenic reactions. Tissue plasminogen activator (t-PA) and single chain urokinase-type plasminogen activator (scu-PA, also called u-PA or simply urokinase) are second-generation agents. Both activators bind avidly to fibrin, enabling them to cause efficient and localized digestion of the clot or thrombus. As seen from results of clinical trials, the high doses required of these agents produce a mild to moderate decrease in levels of fibrinogen and plasminogen, indicating that the fibrin selectivity is

Maribel Diaz-Ricart[1]*, M. Bayés[2]. [1]Servicio de Hemoterapia y Hemostasia, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain; [2]Prous Science, P.O. Box 540, 08080 Barcelona, Spain. *Correspondence.

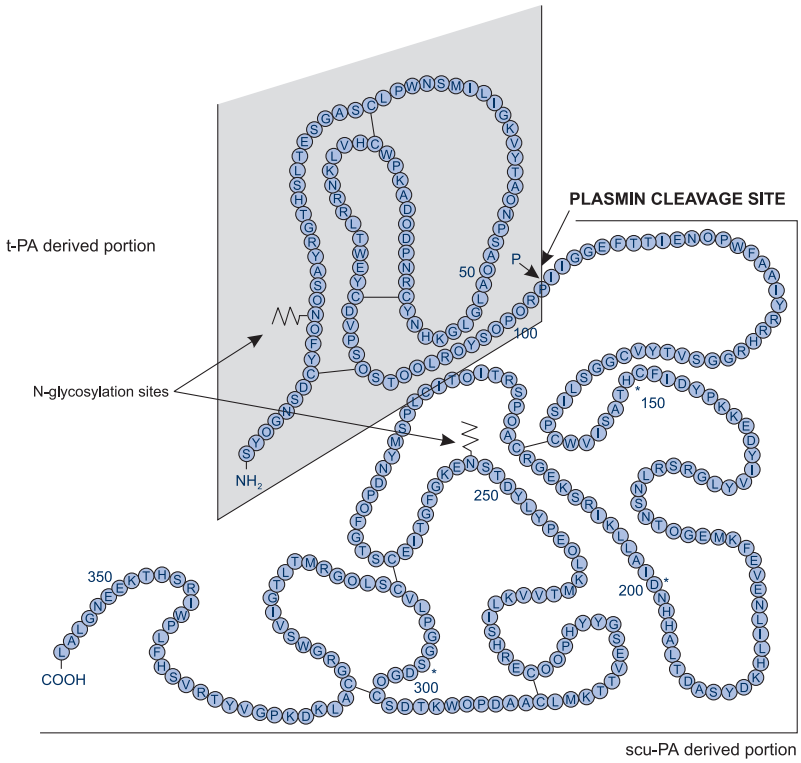

Fig. 1. Amino acid sequence of amediplase. Amediplase is a single-chain glycoprotein composed of amino acids 1-3 and 176-275 of t-PA (kringle-2 domain plus the adjacent linker region to the t-PA B-chain) and amino acids 159-411 of scu-PA, encoding the full length B-chain. *Catalytically significant amino acids of the serine protease active site. (Adapted from ref. 11.)

rather limited and is influenced by the dose and duration of infusion (6, 7).

In recent years, several forms of mutants and hybrids of plasminogen activators have been developed. In the t-PA molecule there are several regions, one of which is responsible for the high-affinity binding to fibrin (fibronectin finger region), another for the binding to receptors in the liver (the epidermal growth factor and the kringle-1 region) and another for the low-affinity binding to fibrin (kringle-2 region). The protease domain is responsible for the cleavage of plasminogen and also binds the plasminogen activator inhibitor type-1 (PAI-1), inhibiting the proteolytic function of t-PA. The asparagine in positions 117, 184, and 448 carries carbohydrate chains that affect the plasma clearance of the molecule via hepatic endothelial cells. Amediplase is one of the third-generation plasminogen activators that have been developed in order to lengthen the half-life of the drug, to increase resistance to plasma protease inhibitors, to improve the specificity for fibrin (8) and to diminish immunogenicity.

Pharmacological Actions

In animal models, amediplase or K_2tu-PA is a more potent and longer-lasting thrombolytic agent than alteplase (rt-PA), the standard thrombolytic drug for the treatment of acute myocardial infarction. Amediplase is indicated for administration as a single bolus, which constitutes an advantage over the standard thrombolytic treatments.

Studies performed *in vitro* (9) indicated that the specific activity of K_2tu-PA, as measured by fibrin plate, was 2.5 million t-PA equivalent units/mg. On a molar basis, K_2tu-PA was at least twice as active as t-PA. Like scu-PA, K_2tu-PA was insensitive to inhibition by plasminogen activator inhibitor-1 (PAI-1). The concentration of K_2tu-PA that produced 50% lysis of blood clots in 3 h was 0.5 μg/ml, as compared to 0.5 and >2 μg/ml for t-PA and scu-PA, respectively. Plasminogen and α_2-antiplasmin consumption induced by K_2tu-PA in clot-free plasma was comparable to that induced by either t-PA or scu-PA. When exposed to plasmin, K_2tu-PA was completely converted into two-chain molecules with full enzymatic activity, although both still required fibrin for full expression of activity. As reported by Colucci *et al.* (9), the absence of one of the t-PA domains involved in rapid hepatic clearance (the kringle-1 domain) markedly improved the pharmacokinetic properties of K_2tu-PA, as demonstrated in rabbits and monkeys. In monkeys, the clearance rate was about 10 times lower than that of t-PA.

Studies *in vivo* comparing the thrombolytic activity of equal concentrations of rt-PA and K_2tu-PA (0.4, 0.8 and 1.2 mg/kg) were performed in a jugular vein thrombosis

Box 1: Amediplase in acute myocardial infarction (13) [from Prous Science Integrity®]

Design	Multicenter, open, crossover, dose-finding, randomized clinical study
Population	Patients with acute myocardial infarction within 6 h from onset of symptoms (n=149)
Treatments	Amediplase, 20-90 mg i.v. bolus + Heparin + Aspirin
Withdrawals	9/149 (6.0%) [no evaluable angiography]
Adverse Events	Serious 32 events, intracranial hemorrhage 1/149 (0.7%), other fatal major bleed 1/149 (0.7%)
Results	Dose that achieved TIMI grade 3 flow >50% @ 90 min: >60 mg; adjusted to body weight: >0.7 mg/kg Death rate @ 30 d: 5/149 (3.4%)
Conclusions	Amediplase was safe and effective in acute myocardial infarction

Box 2: Weight-adjusted doses of amediplase in myocardial infarction (14) [from Prous Science Integrity®]

Design	Multicenter, double-blind clinical study
Population	Patients with acute myocardial infarction within 6 h of symptom onset (n=240)
Treatments	Amediplase, 1.0 mg/kg i.v. bolus Amediplase, 1.2 mg/kg i.v. bolus
Withdrawals	27/240, 11.3% [angiograms not available]
Adverse Events	Major bleeding episodes (3/240, 1.2%), intracranial hemorrhages (2/240, 0.8%)
Conclusions	Preliminary results of the total group suggested that amediplase had an acceptable safety profile and efficacy in patients with myocardial infarction

model in rabbits (10). Results indicated that the thrombolytic activity of K_2tu-PA was significantly greater than rt-PA at the two higher doses evaluated. Both compounds produced a similar reduction of fibrinogen, α_2-antiplasmin and plasminogen, measured 3 h after beginning infusion of the two higher doses. In a further study using the same experimental model (11), both rt-PA and K_2tu-PA were administered as a single bolus at concentrations of 0.2, 0.4 and 0.8 mg/kg. Bolus doses of both compounds produced a similar degree of fibrinolysis. Due to the longer half-life of K_2tu-PA (> 30 min *vs.* 4 min for rt-PA), it was more efficient than rt-PA in inhibiting accretion of new fibrin on the thrombi during thrombolysis and in reducing the thrombus size. The higher dose of K_2tu-PA produced more severe systemic proteolysis and bleeding than the highest dose of rt-PA. However, the authors concluded that the potential increased risk of bleeding with bolus doses of K_2tu-PA has to be seen in view of the advantage of avoiding the concomitant use of heparin. The efficacy of K_2tu-PA has also been demonstrated in a dog coronary artery thrombolysis model (12).

Clinical Studies

The efficacy and safety of amediplase were evaluated in an open-label, multicenter, randomized, dose-finding study. Amediplase was given as a single bolus, in the dose range of 20-90 mg, in patients with suspected acute myocardial infarction treated with heparin and aspirin within 6 h from the onset of symptoms. Efficacy was evaluated by measuring coronary artery patency (TIMI 3 flow grade) by angiography at 90 min after amediplase administration. Flow grade was defined according to the criteria established by the Thrombolysis in Myocardial Infarction (TIMI) Trial investigators as follows: grade 0, a totally occluded artery (no flow); grade 1, flow into the thrombus but not beyond (blood flow occluded); grade 2, sluggish flow through the vessel (still not normal flow); and grade 3, normal brisk flow through the artery. Of the 149 patients randomized to the study, 140 were evaluable for angiographic data. Rescue percutaneous transluminal coronary angioplasty (PTCA) was performed in patients with TIMI 0-2 flow at 90 min. TIMI 3 flow grade at 90 min was obtained in more than 50% of the patients treated with doses higher than 60 mg, corresponding to >0.7 mg/kg when doses were adjusted to body weight. Pharmacokinetic analysis showed a plasma half-life value supporting single bolus dosing. Mortality at 30 days was remarkably low, being 3.4% (5 of 149 patients included in the study). Thirty-two serious adverse events were reported, mostly related to the myocardial infarction. Only one intracranial hemorrhage and one other fatal major bleed were reported. It was concluded that amediplase has a

good safety profile with a low incidence of serious bleeding complications, and that further investigations are needed to assess the optimal dose regimen for the agent (13) (Box 1).

A multicenter, double-blind trial evaluated two weight-adjusted doses of amediplase (1.0 or 1.2 mg/kg) in patients with suspected acute myocardial infarction presenting within 6 h of symptom onset. The primary endpoint of the study was coronary artery patency at angiography, which was performed 60 min after administration of a single bolus dose of amediplase. Of 241 patients enrolled in the study, 213 were evaluable for angiograms. At 60 min after administration 57.9 and 76% of the patients had TIMI grade 3 and TIMI grade 2-3 flow, respectively. The safety profile was acceptable, with 3 cases of major bleeding episodes and 2 cases of intracraneal hemorrhage, 1 of which was fatal. The 30-day overall mortality rate was 4.5% (14) (Box 2).

Source

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