Leading article

New strategies in the development of thrombolytic agents

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Summary. Recombinant DNA technology has allowed large-scale production of the physiological, fibrin-specific, plasminogen activators tissue-type plasminogen activator (t-PA) and single-chain urokinase-type plasminogen activator (scu-PA). The results of clinical trials with these agents, mainly for the treatment of acute myocardial infarction, have revealed a limited fibrin specificity at the large therapeutic doses required for efficient thrombolysis. Mutants and variants of t-PA and scu-PA have given important information on structure-function relationships in these proteins and have resulted in rt-PA variants with significantly prolonged half-lives in vivo. Construction of chimaeric plasminogen activators containing various portions of t-PA and scu-PA has produced functionally active enzymes, however with a lower fibrin-affinity than wild-type t-PA. The promise of antibody targeting and the use of synergistic combinations of thrombolytic agents remains to be further investigated. We anticipate that eventually these research lines will yield artificial plasminogen activators with improved efficacy, risk/benefit and cost/benefit ratios.

Key words: Tissue type plasminogen activator – Urokinase-type plasminogen activator – Chimaeric plasminogen activators – Synergism

Introduction

Thrombotic occlusion of critically situated blood vessels is a common triggering event in the major clinical syndromes of cardiovascular disease. In over 80% of patients with acute myocardial infarction (AMI), coronary artery occlusion due to thrombosis is observed within 4 h of the onset of symptoms [1, 2]. One approach to the treatment of an established thrombosis consists in the administration of plasminogen activators which activate the fibrinolytic system in the blood. The fibrinolytic system (Fig. 1) is composed of an inactive proenzyme, plasminogen, which is converted to an active serine protease, plasmin, by different types of plasminogen activators, including streptokinase (SK), single-chain urokinase-type plasminogen activator or pro-urokinase (scu-PA), two-chain urokinase-type plasminogen activator (tcu-PA), tissue-type plasminogen activator (t-PA) and anisoylated plasminogen-streptokinase activator complex (APSAC). These plasminogen activators may be divided into two main categories: a first generation comprising SK and tcu-PA and a second generation with t-PA, scu-PA and APSAC. SK, u-PA and APSAC activate plasminogen both at the fibrin surface and in the circulation, resulting in extensive generation of plasmin with exhaustion of α2-antiplasmin.

Fig. 1. Schematic representation of the fibrinolytic system. The different plasminogen activators are: streptokinase (SK), two chain urokinase-type plasminogen activator (tcu-PA), single chain urokinase-type plasminogen activator (scu-PA), tissue-type plasminogen activator (t-PA) and anisoylated plasminogen-streptokinase activator complexes (APSAC)
and degradation of fibrinogen and other plasma proteins such as factor V and factor VIII. The physiological plasminogen activators t-PA and scu-PA activate plasminogen more specifically at the fibrin surface (Fig. 2). Plasmin, associated with the fibrin surface, is protected from rapid inhibition by α2-antiplasmin and may thus efficiently degrade fibrin into soluble fibrin degradation products [3].

The physicochemical properties and the mechanism of action of t-PA and scu-PA have previously been described in detail [4]. Clinical experience has revealed that the thrombolytic potency and the fibrin-specificity of these physiological plasminogen activators in man is not as pronounced as was anticipated from studies in animal models [5]. At the high therapeutic doses required for treatment of patients with AMI, the fibrin-specificity is only limited, and hemorrhagic complications may occur. In addition, reocclusion due to residual stenosis following successful reperfusion represents a major problem of thrombolytic therapy. A number of pharmacological means to reduce the incidence of reocclusion are presently being investigated, mainly following treatment with t-PA. These include the administration of a maintenance dose of t-PA [6–8], coronary angioplasty [9–12] and the use of a monoclonal antibody against glycoprotein IIb/IIIa, the platelet fibrinogen receptor [13, 14].

In addition, research into more fibrin-specific thrombolytic agents or strategies continues. Five main lines of research are being explored, including antibody targeting, mutants of scu-PA, mutants of t-PA, chimaeric molecules and synergism between thrombolytic agents.

**Antibody-targeted thrombolytic therapy**

Several alternatives to target the action of thrombolytic agents towards the thrombus with the use of fibrin-specific antibodies are presently being investigated. These include chemical conjugates of fibrin-specific antibodies with urokinase or rt-PA [15–20], or recombinant fusion proteins comprising a fibrin-specific antibody site and the B-chain of t-PA [21].

Bode et al. [17] have linked urokinase covalently to a monoclonal antibody (64C5) specific for the aminoterminus of the β-chain of human fibrin, by means of the cross-linking reagent N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) following introduction of sulfhydryl groups with 2-iminothiolane. Alternatively, the inter-heavy chain sulfhydryl of the Fab' of the antibody was linked to SPDP-substituted urokinase [17, 18]. Both conjugates lysed fibrin monomer with an about 100-fold higher potency than urokinase [15, 18], indicating that the antibody had maintained its fibrin-specificity and that univalent binding to fibrin (Fab’-urokinase) is sufficient to enhance fibrinolysis. The fibrinolytic potency of the Fab’-urokinase conjugate towards cross-linked thrombi in citrated plasma was found to be only 4.4 times that of uncoupled urokinase [18].

Subsequently, the authors have used the same technique to couple single chain t-PA to a monoclonal antibody 59D8 with similar specificity [19, 20]. t-PA was shown to lyse fibrin monomer 10 times more efficiently than urokinase, whereas both urokinase-59D8 and t-PA-59D8 conjugates were 100 times more potent than urokinase alone. The authors have also studied the use of a monoclonal antibody to platelet fibrinogen receptor, which was shown to be effective in preventing reocclusion in an animal model [22].

In summary, the development of fibrin-specific thrombolytic agents is an active area of research, with various strategies being explored to improve the thrombolytic potency and specificity of these agents. Further studies are needed to optimize the design of these agents for clinical use.

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**Fig. 2. Molecular interactions determining the fibrin-specificity of plasminogen activators**