Clinical trial results with a new plasminogen activator

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Thrombolysis has become an accepted form of therapy for acute myocardial infarction. As demonstrated in the Global Utilization of Streptokinase and t-PA for Occluded Arteries trial, early, complete and sustained patency of the infarct-related coronary artery is correlated with reduced mortality. However, current thrombolytic regimens are able to achieve such patency within 90 min in only 81% of cases. To improve the risk/benefit ratio of thrombolytic therapy, newer agents such as reteplase have been developed to establish more rapid, more complete and more stable coronary artery patency, thus reducing mortality.

This report summarizes the pharmacological properties of reteplase. It also summarizes the findings from various animal and clinical studies in which reteplase was compared with alteplase and streptokinase and the findings from animal and clinical studies evaluating infusion, single-bolus, and double-bolus doses of reteplase.

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Introduction

Thrombolytic therapy has become an accepted form of treatment for acute myocardial infarction (MI). The Global Utilization of Streptokinase and t-PA for Occluded Arteries (GUSTO) trial showed that mortality reduction correlates with early, complete and sustained patency of the infarct-related coronary artery. With current thrombolytic regimens, patency at 90 min after initiation of treatment is achieved in only about 81% of cases, and only about 54% of patients experience complete (TIMI grade 3) reperfusion; earlier patency rates are even more disappointing. Early re-occlusion further limits the preservation of left ventricular function. In addition, even though patients are carefully selected, bleeding — especially intracranial bleeding — is a feared side effect, limiting the applicability of this form of treatment. To improve the risk/benefit ratio of thrombolytic therapy for patients, new thrombolytic agents are being developed to reduce mortality by establishing more rapid, more complete and more stable coronary patency. This review summarizes the molecular characteristics and the pharmacological properties of reteplase obtained from in vitro and experimental animal studies, as well as from clinical studies evaluating patency and mortality. Although most studies of reteplase have expressed doses in mega-units (MU), on the basis of an amidolytic assay, doses in this review are cited in units (U) based on a new clot lysis assay.

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Molecular characteristics of reteplase

Reteplase, which is alternatively referred to as recombinant plasminogen activator (r-PA) or BM 06-022, is a genetically engineered deletion variant of human tissue plasminogen activator (t-PA). Reteplase is produced by expression of an appropriately constructed plasmid in Escherichia coli, where it is localized in inclusion bodies. As with many proteins expressed in prokaryotic cells, the fully functional, non-glycosylated protein becomes available after an in vitro refolding process.

SDS-polyacrylamide electrophoresis and amino acid analysis of reteplase reveal a single-chain, non-glycosylated protein of 39-6 kd, which consists of amino acids 1 to 3 and 176 to 527 of human t-PA. Reteplase consists of the kringle 2 and the protease domains of human t-PA; the kringle 1, finger and epidermal growth factor domains have been deleted. The one-chain form can be cleaved by plasmin to a two-chain form. The structural changes relative to human t-PA result in markedly different properties in vitro and in vivo.

Reteplase: in vitro studies

The structure and function of the enzymatic domain of native human t-PA are largely retained by reteplase, which has been shown to have low fibrin binding, although it is fibrin-specific in vivo at appropriate doses. While the plasminogenolytic activity of reteplase is similar to that of alteplase (rt-PA) in the absence of stimulatory CNBr fibrinogen fragments,
reteplase's activity is about 4-fold less, when compared on a molar basis in the presence of these stimulatory fragments[3]. PAI-1 inhibition was similar for reteplase and for rt-PA, indicating that the responsible structures of the kringle 2 and the protease domains were identical in the two molecules[4]. A lower affinity of r-PA for endothelial cells, compared with rt-PA, has been reported[3], presumably because reteplase is not glycosylated and/or because it lacks finger and growth factor domains. Extensive studies of in vitro lysis of fresh, aged, platelet-poor, platelet-rich and whole blood clots have been performed[6]. To achieve 50% clot lysis 4 h into the experiment, the investigators had to use 6-4 times higher molar concentrations of reteplase vs alteplase. The data suggest that — in vitro — reteplase achieved a lower thrombolytic potency, especially in lysing aged and platelet-rich clots when compared to alteplase on a molar basis. In further experiments, clots incubated with reteplase or alteplase were transferred to plasminogen-activator-free plasma. Clot lysis continued for 3 h with alteplase. In contrast, no further lysis occurred with reteplase. These differences may well be of interest in a clinical situation.

Reteplase in experimental animals

The intended prolongation of reteplase's half-life relative to rt-PA could be demonstrated in rats, dogs and non-human primates[7]. In the rabbit model of jugular vein thrombosis, reteplase proved to be 5-3 times more effective than alteplase when both activators were given as a bolus. Plasma clearance of reteplase was 4-3-fold lower than that of alteplase; thus the apparent higher potency of reteplase may well be due to its lower clearance rate. At equipotent dosages, residual fibrinogen was similar for both activators. Thus, no relative loss in specificity was observed[9].

In a canine model of coronary thrombosis, reteplase was compared with alteplase, anistreplase, urokinase and streptokinase. Reperfusion was achieved significantly more rapidly with reteplase than with the other tested plasminogen activators and, remarkably, bleeding time was least affected[9]. To achieve 50% reperfusion, the intravenous reteplase dose required was 11-6-fold lower than the intravenous alteplase dose in another study using a dog model of coronary thrombosis[10]. Further experiments suggest that an antithrombotic adjunct may be useful to preserve patency after fibrinolysis with reteplase[11,12]. Double-bolus administration of reteplase was shown to be more effective than infusion or single-bolus regimens[13].

Reteplase also proved useful in reversing pulmonary hypertension in a canine model of pulmonary embolism. Because of its bolus application, reteplase acted more quickly than did other plasminogen activators in these experiments[14]. Reteplase and alteplase had no effect on platelet count or, when given alone, on ex vivo platelet aggregation. In animals pretreated with aspirin, reteplase significantly reduced platelet aggregation as compared with alteplase[15], a difference that was not observed in patients[16].

Clinical studies with reteplase

Phase 1 studies evaluating the effects of reteplase on healthy volunteers provided no data to preclude phase II testing[17,18]. The first phase II trial with reteplase was designed as an open, sequential, dose-finding study in patients with acute M1[9]. With the first-tested dose of 10 U of reteplase, the predefined minimal 90-min patency of 70% was not achieved, as indicated by a sequential probability ratio test after treatment of 42 patients. The 90-min patency rate (TIMI 2 or 3) was 67%. An increased dose of 15 U reteplase administered as a single bolus resulted in a patency at 90 min (TIMI 2 or 3) of 76% in the subsequent 100 patients (74% at 60 min). Complete patency (TIMI 3) after 90 min was achieved in 69% of patients in the 15 U group and in 52% of those in the 10 U group. To increase efficacy further, the concept of double-bolus administration was investigated in the second German Recombinant Plasminogen Activator study (GRECO 2), an open, non-controlled, dose-finding study involving 50 patients. TIMI 2 or 3 patency at 60 and 90 min after a double-bolus regimen (10 U+5 U, 30 min apart) was 72% and 78%, respectively, and TIMI 3 patency was 50% and 58%[19].

The 15 U bolus regimen, the 10 U+5 U double-bolus regimen, and a new 10 U+10 U regimen of reteplase were compared to conventional alteplase (100 mg over 3 h) in the Reteplase Angiographic Phase II International Dose-Finding Study (RAPID 1), a randomised study involving 606 patients with acute M1[20]. The 10 U+10 U reteplase regimen was superior to the other reteplase regimens and also achieved better 60- and 90-min TIMI 3 patency than did alteplase (reteplase vs alteplase at 60: 51-0% vs 32-7%, P<0.01; and at 90 min: 62.7% vs 49.3%, P<0.05).

Similarly, overall patency (TIMI 2 and 3) was better after treatment with reteplase 10 U+10 U, although not significantly so (reteplase vs alteplase at 60 min: 77-6% vs 66-3%; and at 90 min: 85-2% vs 77-8%). Patency was achieved more rapidly with reteplase than with alteplase. TIMI 3 patency at 60 min for reteplase (51%) was similar to that for alteplase at 90 min (49.3%). Superior speed and completeness of reperfusion resulted in better preservation of left ventricular function in the 10 U+10 U reteplase group[22]. The risk of bleeding was not significantly different for reteplase and conventional alteplase[23]. There was no significant difference between reteplase and alteplase with respect to platelet aggregation or thrombin activity during treatment and up to 12 h after treatment of patients with acute M1[24].

In an effort to assess safety and efficacy in a larger study population, the International Joint Efficacy Comparison of Thrombolics (INJECT) study was performed[24]. Reteplase 10 U+10 U was compared to
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Figure 1 Patency rates at different time points achieved with alteplase (rt-PA) and reteplase (r-PA). □ Refers to TIMI 2; □ refers to TIMI 3; * Denotes P<0.05 for r-PA vs rt-PA; ** denotes P<0.01 for r-PA vs rt-PA. (Adapted from Bode et al.)

streptokinase (SK) in 6010 patients with acute MI in a double-blind, randomised trial with 35-day mortality as the primary endpoint. The primary aim of the study was to demonstrate that the 10 U + 10 U double-bolus regimen of reteplase is at least equivalent to the standard regimen of SK in terms of mortality. Equivalence was defined as a 35-day mortality rate for reteplase of not more than 1% higher than for SK. The trial was not powered to demonstrate superiority and had design features (e.g. recruitment up to 12 h, trial confined to Europe) that made it difficult to compare it to other trials, such as GUSTO I.

Death up to 35 days after the index infarction occurred in 9-02% of 2994 patients in the reteplase group and in 9-53% of 2992 patients in the SK group. The difference of −0-51% (90% confidence interval [CI] −1-74% to 0-73%) in favour of reteplase was not statistically significant. Thus, the efficacy of reteplase was shown to be equivalent to that of SK according to the above-stated definition. Mortality at 6 months was also equivalent, with 11-02% in the reteplase group and 12-05% in the SK group (difference −1-03%; 90% CI −2-65% to 0-59%). Patient follow-up was 99-6% complete. The incidence of cardiac shock (4-7% vs 6% for reteplase vs SK), heart failure (23-6% vs 26-3%), hypotension (15-5% vs 17-6%), and atrial fibrillation (7-2% vs 8-8%) was significantly lower in the reteplase group.

The INJECT study also provided comparative data on safety and tolerability. The overall incidence of bleeding (15% vs 15-3% for reteplase vs SK) and significant bleeding (4-6% vs 4-7%) was similar in both groups. In addition, there was a small non-significant excess of in-hospital strokes in the reteplase group compared to streptokinase group (1-23% vs 1%). The incidence of haemorrhagic strokes, however, was higher for reteplase than for streptokinase (0-77% vs 0-37%). A similar increase in the incidence of intracranial haemorrhage was also observed for alteplase compared with streptokinase (0-72% vs 0-54%) in the GUSTO-I trial. Data from the invasive RAPID 1 and 2 studies support these findings in showing no excess side effects for reteplase vs alteplase.

After the GUSTO study had shown that an accelerated dose regimen for alteplase (100 mg over 90 min) was superior to SK (and possibly also to conventional alteplase therapy with respect to efficacy and safety), the RAPID 2 study was undertaken. The 10 U + 10 U reteplase regimen was compared to the GUSTO regimen of alteplase in an open, randomised trial enrolling 324 patients. The study design of RAPID 2 differed from RAPID 1 in that there was no age limit (RAPID 1 <75 years) and patients were included up to 12 h after onset of pain (RAPID 1 <6 h). For both RAPID 1 and RAPID 2, coronary angiograms were reviewed in a blinded fashion by a centrally located evaluation team.

In the RAPID 2 study, infarct-related coronary artery patency (TIMI 2+3) and complete patency (TIMI 3) at 90 min were significantly higher in the reteplase-treated patients (TIMI 2+3: 83-4% vs 73-3% for reteplase vs alteplase; P<0.03; TIMI 3: 59-9% vs 45-2%, P=0-01). At 60 min, the incidence of both patency and complete patency was also significantly higher in reteplase-treated patients (TIMI 2+3: 81-8% vs 66-1%; P=0-01; TIMI 3: 51-2% vs 37-4%, P=0-03) (Fig. 1). Reteplase-treated patients required fewer additional acute coronary interventions (13-6% vs 26-5%, P<0.01), and there was no apparent increase in death (reteplase vs alteplase: 4-1% vs 8-4%), stroke (1-8% vs 2-6%), haemorrhagic stroke (1-2% vs 1-9%), re-occlusion (9-0% vs 7-0%), or bleeding rate (12-4% vs 9-7%) (Fig. 2)28.
The GUSTO III study is investigating whether the apparent advantages of reteplase in achieving early and complete patency will translate into a further decrease in mortality.

Implications for clinical practice and future research

Double-bolus administration (10 U + 10 U) of reteplase thus results in effective, rapid and complete lysis of coronary thrombi in the majority of patients. With respect to these parameters, the 10 U + 10 U regimen was superior to established alteplase regimens in the RAPID studies. Furthermore, from the INJECT study, patients treated with reteplase had a significantly lower incidence of atrial fibrillation, asystole, cardiogenic shock, CHF and hypotension than patients treated with streptokinase. On the basis of the results of the GUSTO I trial and its angiographic sub-study, there is reason to believe that the GUSTO III study will demonstrate that improved patency will translate into clinical benefit. Patency appears to be an appropriate surrogate endpoint for clinical outcome, but only when investigated in large, multi-centre, randomized trials.

That these requirements appear mandatory may be extrapolated from two examples. Double-bolus administration of alteplase was first investigated in a relatively small, single-centre, non-randomized study, suggesting very high patency rates that are superior to those achievable with an accelerated alteplase infusion. In a larger study, the alteplase double bolus in fact turned out to be slightly less effective than the accelerated alteplase infusion. A similar pattern could be noted when the GUSTO IIb investigators showed patency results for acute percutaneous transluminal coronary angioplasty (PTCA) that were far inferior to those previously reported in smaller trials. In fact, the patency rates for reteplase-treated patients recruited within 6 h in the RAPID 2 study (TIMI 2 + 3: 86.5%; TIMI 3: 65%) are closer to those reported for acute PTCA in the GUSTO IIb trial (TIMI 2 + 3: 82%; TIMI 3: 75%) than to those of other thrombolytic regimens. Thus, a large trial comparing prehospital thrombolysis with double-bolus reteplase (exploiting this logistic advantage of thrombolysis) to acute PTCA would be interesting.

New thrombolytic agents, such as antibody-targeted plasminogen activators that have been produced as conjugates and recombinant molecules, appear promising and may further improve thrombolytic therapy. Potency and specificity of these agents compare favourably to conventional plasminogen activators in different animal models. More specific thrombolytic agents that leave the clotting system largely intact may well require equally specific anticoagulants as adjuncts.

Although other new approaches are on the horizon, reteplase, which offers the convenience of a double-bolus injection, has been proven at present to be an extremely potent and safe thrombolytic agent in patients with acute MI. If the GUSTO III study substantiates the clinical results demonstrated thus far, reteplase may well become the standard against which other thrombolytic agents and mechanical reperfusion strategies will be compared to prove their usefulness. Continuous advances in mechanical strategies (e.g. newer stents) and possible combinations of both thrombolytic agents and mechanical 'rescue' strategies deserve further attention. The addition of 'platelet-passivating' agents such as GP IIb/IIIa receptor blockers may improve the outcome of thrombolytic, mechanical, and combined strategies.

References


