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Off-target effects of thrombolytic drugs: apolipoprotein A-I proteolysis by alteplase and tenecteplase

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ABSTRACT

The administration of thrombolytic drugs is of proven benefit in a variety of clinical conditions requiring acute revascularization, including acute myocardial infarction (AMI), ischemic stroke, pulmonary embolism, and venous thrombosis. Generated plasmin can degrade non-target proteins, including apolipoprotein A-I (apoA-I), the major protein constituent of high-density lipoproteins (HDL). Aim of the present study was to compare the extent of apoA-I proteolytic degradation in AMI patients treated with two thrombolytic drugs, alteplase and the genetically engineered t-PA variant tenecteplase. ApoA-I degradation was evaluated in sera from 38 AMI patients treated with alteplase or tenecteplase. In vitro, apoA-I degradation was tested by incubating control sera or purified HDL with alteplase or tenecteplase at different concentrations (5–100 μg/ml). Treatment with alteplase and tenecteplase results in apoA-I proteolysis; the extent of apoA-I degradation was more pronounced in alteplase-treated patients than in tenecteplase-treated patients. In vitro, the extent of apoA-I proteolysis was higher in alteplase-treated sera than in tenecteplase-treated sera, in the whole drug concentration range. No direct effect of the two thrombolytic agents on apoA-I degradation was observed. In addition to apoA-I, apoA-IV was also degraded by the two thrombolytic agents and again proteolytic degradation was higher with alteplase than tenecteplase. In conclusion, this study indicates that both alteplase and tenecteplase cause plasmin-mediated proteolysis of apoA-I, with alteplase resulting in a greater apoA-I degradation than tenecteplase, potentially causing a transient impairment of HDL atheroprotective functions.

1. Introduction

The administration of thrombolytic drugs is of proven benefit in a variety of clinical conditions requiring acute revascularization, including acute myocardial infarction (AMI), ischemic stroke, pulmonary embolism, and venous thrombosis. Thrombolytic drugs cleave fibrin clots by activating plasminogen to the active enzyme plasmin which acts by breaking down fibrin in the clot to soluble degradation products. In the case of AMI, the goal of therapy with thrombolytic agents is the restoration of blood flow through the coronary artery occluded by the thrombus. The rationale for this approach is the reduction of myocardial necrosis and greater preservation of myocardial function with the objective of reducing mortality and associated morbidity [1]. The utility of thrombolytic therapy in acute myocardial infarction was originally demonstrated with streptokinase infusions [2]. The benefits of streptokinase in reducing mortality, however, are tempered by its immunogenicity and its lack of fibrin specificity, which led to the development of new compounds, the most notable of which is alteplase, the recombinant form of tissue plasminogen activator (t-PA) [3]. Although alteplase reduces mortality associated with acute myocardial infarction, its application is limited by the necessity of prolonged intravenous infusion regimen, which stimulated the development of new agents with more favorable pharmacokinetics. The modifications to t-PA to produce tenecteplase included Asn-103 for Thr and Gln-117 for Asn substitutions, modifying two glycosylation sites, and substitution of residues 296-299 with four alanines, conferring enhanced fibrin specificity and resistance to PAI-1 inhibition [3]. These changes conferred a prolonged half-life, allowing administration of the drug as a single bolus; in addition, its improved fibrin-specificity resulted in a reduced systemic plasmin activation [3].

Abbreviations: AMI, acute myocardial infarction; apoA-I, apolipoprotein A-I; BMI, body mass index; CK, creatine kinase; CK-MB, CK myocardial band; CRP, C-reactive protein; HDL, high density lipoproteins; LDL, low density lipoproteins; RCT, reverse cholesterol transport; TnI, Troponin I; t-PA, tissue plasminogen activator.

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Apolipoprotein A-I (apoA-I), the main protein component of high-density lipoproteins (HDL), has been shown to be sensitive to a series of proteases, including plasmin [4–6]. A previous observation from our group showed, in a small group of patients with AMI, that apoA-I degradation can occur during tenecteplase therapy [7], possibly related to the pharmacologic agent itself or to the resulting "lytic" state. In the present study, the effect on apoA-I degradation of two thrombolytic agents with different fibrin-specificity, alteplase and tenecteplase, was evaluated in AMI patients undergoing fibrinolysis. In addition, in vitro experiments were carried out to verify the direct effect of the two drugs on apoA-I degradation.

2. Materials and methods

2.1. Patient selection

Thirty-eight patients presenting with ST-elevation myocardial infarction (STEMI) and undergoing thrombolytic therapy were selected among a previously described cohort [8]. Nineteen patients were treated with alteplase (intravenously bolus of 15 mg + 0.75 mg/kg continuous infusion for 30 min + 0.5 mg/kg continuous infusion for 60 min if <65 kg, or intravenously bolus of 15 mg + 50 mg continuous infusion for 30 min + 35 mg continuous infusion for 60 min if >65 kg) and 19 with tenecteplase (intravenously bolus of 0.53 mg/kg). All patients received aspirin, morphine, sedatives, nitrates and beta-blockers if haemodynamically tolerated; heparin regimen was standard (intravenously bolus of 60 U/kg + 12 U/kg per hour continuous infusion).

Blood was collected at admission and after 4, 8, 12, 24, 48, 72, and 96 h, and at hospital discharge; in the present study, samples taken at admission and after 4 h were analyzed. The study was approved by the local institutional Ethic Committee, and all enrolled patients gave written informed consent for participation in the study.

2.2. Biochemical analyses

Fasting blood samples were collected, serum and plasma were prepared by low-speed centrifugation. The plasma concentrations of total, LDL- and HDL-cholesterol, triglycerides, apoA-I, apoA-II and apoB were determined by standard techniques as described (Roche Diagnostics, Mannheim, Germany) [8]. The extent of the inflammatory response induced by AMI was evaluated by measuring plasma levels of C-Reactive Protein (CRP) from admission to discharge (Roche Diagnostics) [8]. The severity of AMI was evaluated by measuring the plasma concentrations of creatine kinase (CK), CK-myocardial band (CK-MB) and Troponin I (TnI).

2.3. Effect of thrombolytic drugs on apoA-I degradation in vitro

For in vitro studies, serum was collected from five healthy volunteers (Controls). HDL (d = 1.063–1.21 g/ml) were isolated by sequential ultracentrifugation, dialyzed against saline immediately before use, and diluted at the physiological concentration of 1 mg of protein/ml. Control sera and isolated HDL were incubated at 37 °C with 5–100 μg/ml of alteplase or tenecteplase (Boehringer Ingelheim, Ingelheim am Rhein, Germany) for 4 h, a time previously shown to allow the maximal degradation of apoA-I both in vitro and in vivo [7]. Selected alteplase and tenecteplase concentrations ranged from peak plasma levels (4–12 μg/ml [9]) to values ten-fold higher. For time-course experiments, Control sera were incubated with 100 μg/ml of alteplase or tenecteplase up to 8 h.

2.4. Evaluation of apoA-I degradation

Serum and HDL proteins were separated by SDS-polyacrilamide gradient gel electrophoresis (PAGE), electroblotted on a nitrocellulose membrane (Whatman, Dassel, Germany) and developed with antibodies against human apoA-I (Rockland Immunochemicals, Gilbertsville, PA, USA), apoA-II (Calbiochem, Darmstadt, Germany), apoA-IV (kindly provided by Prof. F. Kronenberg [10]), and apoE (Calbiochem). The intensities of intact apoA-I band, and of the 22 kDa and 15 kDa apoA-I fragments were determined by densitometric analysis with a GS-690 Imaging Densitometer and a Multi-Analyst software (Bio-Rad Laboratories, Hercules, CA, USA), and expressed as percentage of total apoA-I signal.

Serum lipoproteins were separated by non-denaturing two-dimensional (2D) electrophoresis, in which agarose gel electrophoresis (separation according to surface charge; Sebia, Lisses, France) was followed by non-denaturing PAGE (separation according to particle dimension) and immunoblotting against apoA-I; this technique allows the separation of HDL in two subpopulations migrating in α position (spherical particles) and preβ position (discoidal particles) [11]. Serum content of preβ-HDL was calculated as percentage of total apoA-I signal; the absolute plasma preβ-HDL concentrations (in mg/dl) were calculated multiplying plasma apoA-I levels by the preβ-HDL percentage values.

2.5. Statistical analysis

Results are reported as mean ± SD, if not otherwise stated. Group differences in continuous variables were evaluated by one-way ANOVA, with post hoc analysis by the Neuman–Keuls test. For categorical variables, group differences were examined with the use of 2×2 contingency tables and a χ^2 test of significance. Group differences with a P value <0.05 were considered statistically significant.

3. Results

3.1. ApoA-I degradation in patients treated with alteplase or tenecteplase

The clinical features and lipid profile at admission of the 19 patients treated with alteplase and the 19 patients treated with tenecteplase are reported in Table 1. None of the patients suffered reinfarction or died during the hospital stay and none had bleeding complications. The two groups were comparable for gender, age, and extent of cardiac enzyme release; groups were also comparable for the extent of the acute inflammatory response, as indicated by plasma CRP levels (Table 1). No differences were detected in the lipid profile at admission, except for higher plasma levels of triglycerides in the tenecteplase group (Table 1).

No evidence of apoA-I degradation was observed in serum samples collected at admission (data not shown); when samples collected after 4 h were analyzed, apoA-I degradation was detected in 9 out of 19 alteplase-treated (47%) and in 7 out of 19 tenecteplase-treated (37%, P = 0.739 vs. alteplase-treated) patients (Table 1). Overall, 16 out of 38 AMI patients treated with thrombolytic agents were found positive to apoA-I degradation. The sensitivity to apoA-I degradation was not associated to differences in gender, age, severity of AMI and extent of the inflammatory response (Table 2). Total and LDL-cholesterol, HDL-cholesterol, apoB and triglyceride levels were similar in the two groups; plasma apoA-I levels were significantly higher in patients negative to apoA-I degradation (Table 2). The percentage and the absolute content of preβ-HDL was also similar in the two groups (Table 2).