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Inhibition of carboxypeptidase U (TAFIa) activity improves rt-PA induced thrombolysis in a dog model of coronary artery thrombosis

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Abstract The objective of this study was to test the hypothesis if thrombolysis induced by recombinant tissue-type plasminogen activator, (rt-PA) could be facilitated by inhibiting carboxypeptidase U (CPU, active Thrombin Activatable Fibrinolysis Inhibitor, TAFIa) activity. The efficacy of rt-PA alone, or in combination with the carboxypeptidase inhibitor MERGETPA, was compared in a dog model of coronary artery thrombosis. Twenty dogs were randomised in two groups, one received rt-PA, 1 mg kg^{-1}, as intravenous infusion over 20 min starting 30 min after thrombus formation, and the other group received rt-PA, 1 mg kg^{-1}, as group one with the addition of MERGEPTA 5 mg kg^{-1} starting 25 min prior to coronary artery occlusion and followed by infusion of 5 mg kg^{-1} h^{-1} until the end of experiment. Efficacy was assessed by determination of time to lysis, duration of patency and blood flow during patency. Both groups had similar baseline characteristics with respect to haemodynamic parameters, i.e., heart rate, blood pressure and coronary artery blood flow. Coadministration of rt-PA and MERGETPA resulted in significant decrease in time to lysis (15±1.5 min vs. 20±1.7 min, p=0.03), increased patency time (87±16 min vs. 46±12 min, p=0.047) and increased coronary blood flow during patency (1131 mL h^{-1} vs. 405 mL h^{-1}, p=0.015), compared to rt-PA alone. These results indicate that an inhibitor of CPU activity may have a beneficial effect in patients undergoing thrombolytic therapy by attaining shorter time to reperfusion and improved coronary patency.

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Introduction

Although thrombolytic therapy increases survival of patients with acute myocardial infarction, rapid and sustained reperfusion with tissue-type plasminogen activator (rt-PA) or streptokinase is achieved only in approximately 50% of the patients [1]. One potential mechanism leading to an early reocclusion might be increased generation of thrombin during thrombolytic therapy [2] or release of active thrombin from the lysed thrombus [3]. Another conceivable mechanism was described by Redlitz et al. [4], who in a dog model of coronary artery thrombosis demonstrated a significant increase of inducible carboxypeptidase activity in plasma that was associated with a prolonged time for restoration of blood flow after treatment with rt-PA. These results were later confirmed by Mattsson et al. [5], and the induced carboxypeptidase activity was identified as carboxypeptidase U (CPU, EC 3.4.17.20) [6,7], also known as activated Thrombin Activatable Fibrinolysis Inhibitor (TAFIa) [8]. In this dog model of coronary artery thrombosis, it was demonstrated that CPU was generated both when the thrombus was formed and when it was lysed by rt-PA. It was therefore hypothesised that the zymogene proCPU (TAFI) was activated by thrombin generated during thrombus formation and possibly also by thrombin released from the lysed thrombus [5].

CPU attenuates fibrinolysis by catalyzing the removal of C-terminal lysine and arginin residues present in partially degraded fibrin. These C-terminal lysine residues are considered to be binding sites for both t-PA and plasminogen thereby promoting local plasmin generation at the site of the thrombus [9—12]. Supporting this mechanism of action for CPU, it was recently demonstrated that a CPU inhibitor isolated from potato tubers (PTI) facilitated rt-PA induced thrombolysis in vivo, using a rabbit thrombolysis model with an experimental thrombus in the renal artery [13]. An improved fibrinolytic effect has also been demonstrated in a dog model of arterial coronary thrombosis when rt-PA was co-administered with the direct thrombin-inhibitor melagatran [14]. This effect of melagatran was probably indirect, i.e., via inhibition of thrombin-mediated activation of proCPU. Thus, it is likely that facilitated thrombolysis can be obtained either by direct inhibition of CPU or indirectly via inhibition of thrombin activity and thereby decreased CPU formation.

The aim of the present investigation was to study the profibrinolytic effect of an inhibitor of CPU in a dog model of coronary artery thrombosis. For this purpose, we used the same dog model as the one used in the earlier thrombin-inhibitor experiments, showing inhibition of CPU generation [5]. Administration of MERGETPA was started before thrombus induction and continued through the whole experiment. This regime was chosen in order to mimic a situation where a CPU inhibitor is used for prophylactic purposes, i.e., the CPU inhibitor is on board already when the thrombus starts to form. The commercially available compound DL-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid (MERGETPA) was used as an inhibitor of carboxypeptidase activity. MERGETPA is an inhibitor of both CPU and carboxypeptidase N (CPN) activity [15—17], and it has been demonstrated that MERGETPA exerts a marked profibrinolytic effect in vivo in a rat model with endotoxin-induced DIC [18]. The mechanism for this effect is most likely due to inhibition of CPU and MERGETPA was therefore considered to be a relevant pharmacological tool in the present study.

Materials and methods

Surgical procedures and instrumentation

This study was approved by the Ethical Committee for Animal Research at the University of Gothenburg, Sweden, and conducted in accordance with the guidelines established in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Twenty (20) beagle dogs of both sexes were used in the study. The rt-PA group included six male and four female dogs with average body weight of 14.1±0.3 kg and the rt-PA+MERGETPA group consisted of five male and five female dogs with an average body weight of 14.6±0.6 kg.

The dogs were anaesthetized with sodium methohexital, 50 mg kg^{-1}, (Brietal, Lilly, Indianapolis, IN, USA) followed by 100 mg kg^{-1} of α-Chloralose (Aldrich-Chemie, Steinheim, Germany). A stable plane of anaesthesia was maintained throughout the experiment by a continuous infusion of α-Chloralose (40 mg kg^{-1} h^{-1}). Oesophageal temperature was monitored and kept maintained within 37±0.5 °C with a thermal table and a heat lamp. After induction of anaesthesia, the dogs were intubated with an end-tracheal cuff tube and ventilated with room air supplemented with 10% oxygen (AGA, Gothenburg, Sweden) by means of a positive-pressure respirator (Servo Ventilator 900C, Siemens Elema, Solna, Sweden). The respiratory rate was held constant at 15 cycles/min. Before