

# Inhalation of single chain urokinase plasminogen activator in pigs exposed to severe gunshot trauma

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## ABSTRACT

The study was carried out at the Department for Thrombosis Research, University of Southern Denmark, and at the Department of Clinical Chemistry, Ribe County Hospital, Esbjerg, in cooperation with the Army Combat School, Oksbøl. The purposes of the study were 1) to investigate whether recombinant single chain urokinase plasminogen activator (rscu-PA) can be aerosolised in a form allowing it to reach the lower respiratory tract without loss of enzymatic activity; 2) to investigate the effects of a severe gunshot trauma on the systemic and alveolar haemostatic balance; 3) to investigate the systemic and alveolar effects on the haemostatic balance of inhaled, aerosolised rscu-PA in severely traumatised pigs. The mass median diameter (MMD) of aerosolised rscu-PA was measured by laser diffraction; the most optimal MMD was 2.96 Fm by jet nebulisation (Ventstream) and 3.69 Fm by ultrasonic nebulisation (Syst-Am DP 100), thus allowing lower respiratory tract deposition. However, ultrasonic nebulisation resulted in a significant loss of enzymatic activity. The outputs from the two types of nebulisers were determined by absorption on filters and found to be similar. The double blind placebo controlled intervention study involved 35 anaesthetised pigs, which were traumatised with a gunshot trauma to one hind leg fracturing the femoral bone. The standardised energy deposition in the tissue had a median of 27.6 J/kg. The animals were maintained on respirators for 48 hours with intervention at 24 hours. The trauma induced a decrease in platelet count, tissue factor and protein C. Antithrombin decreased only transiently. Activated partial thromboplastin time, prothrombin time and fibrinogen increased, and soluble fibrin increased transiently. Thus, a systemic procoagulant state was induced. Analysis of bronchoalveolar lavage fluid showed depressed fibrinolytic activity in the placebo animals. In the treatment animals significantly increased levels of u-PA antigen, u-PA activity, and plasmin specific fibrinolytic activity were found compared with placebo animals, thus confirming the deposition of rscu-PA in the lower respiratory tract, and indicating a shift back to the normal profibrinolytic state in the alveoli. No systemic increase in fibrinolytic activity was recorded. An attempt to estimate the surface area of the alveolar wall covered with fibrin depositions by stereology failed to show any difference between the two groups, while the postmortem macroscopic appearance of the lungs seemed to be different. These results suggest that reversal of the depressed fibrinolytic activity in the alveoli following severe trauma, by inhalation of aerosolised rscu-PA may be a potential preventive or therapeutic approach to the treatment of trauma-induced lung injury. Future studies need to determine the safety of this approach, as well