Recombinant production of a hybrid plasminogen activator composed of surfactant protein B and low-molecular-weight urokinase.

Abstract:
Intraalveolar fibrin deposition is commonly observed during acute inflammatory and chronic interstitial lung diseases and may contribute to impairment of surfactant function and gas exchange. We recently described a chemically cross-linked chimeric protein consisting of surfactant protein (SP)-B and urokinase (uPA) for targeting alveolar fibrin under conditions such as acute respiratory distress syndrome (ARDS) or lung fibrosis. We now investigated the feasibility of a recombinant production of a fusion protein encoding mature SP-B and uPA, termed SPUC. Four different SPUC proteins (N-term SP-B/C-term uPA, N-term uPA/C-term SP-B, each +/- His-tag) were prepared by cloning the cDNA encoding mature SP-B and low-molecular-weight uPA into the expression vector pcDNA3.1. CHO-cells were transfected with the constructs and the supernatant and cell lysates were analyzed for expression of SPUC. Using a chromogenic substrate assay uPA activity was found in supernatants and lysates of transfected cells with highest activities related to the N-term uPA/C-term SP-B (+/- His-tag) construct in supernatants 48h after transfection. Casein enzymography showed an enzymatically active fusion proteins with a molecular weight of approximately 42 kDa in the supernatant of cells transfected with the N-term uPA/C-term SP-B (+/-
His-tag) construct, but only a minor activity with the N-term SP-B/C-term uPA construct. The N-term uPA/C-term SP-B construct was also shown to possess higher resistance towards inhibition by plasminogen activator inhibitor-1. We conclude that recombinant production of a fusion protein consisting of mature SP-B and uPA is feasible, when the SP-B moiety is fused to the C-terminus of urokinase.