

INHIBITING THE INHIBITOR: WOULD TARGETING PAI-1 RESULT IN A LOW-DOSE, WELL-TOLERATED TREATMENT OF EMPYEMA?

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ABSTRACT

The incidence of empyema has continued to rise over the past several decades, and mortality rates remain up to 20-27% among patients >65 years old with comorbidities. Up to 30% of adult patients with empyema are poor candidates for both surgical treatment and conventional fibrinolytic therapy. Plasminogen activator inhibitor 1 (PAI-1) inhibits fibrinolysis, promoting pleural fibrosis and lung restriction. We hypothesized that targeting PAI-1 would result in a low-dose pharmacological treatment for empyema, improving patient survival. This hypothesis was tested in rabbit models of chemically-induced and *Streptococcus pneumoniae* infectious pleural injury. Anti-PAI-1 monoclonal antibodies (mAbs) and a short Docking Site Peptide (DSP), which affect the PAI-1 mechanism differently, were first tested in a rabbit model of chemically-induced pleural injury. Modulating different steps of the PAI-1 mechanism resulted in an up to eight-fold increase in the efficacy of exogenous fibrinolysins. DSP, subsequently tested in a rabbit model of empyema, increased the efficacy of sctPA in both early- and advanced-stage empyema by at least eight and four-fold, respectively. While small-molecule PAI-1 inhibitors are under investigation, there is currently no FDA-approved PAI-1-targeted treatment. We believe that DSP and phage-display selected peptides that compete with mAbs for PAI-1 will be well-tolerated, tractable in clinical settings, and address both pleural fibrosis and thickening with minimal off-target effects. The results support our hypothesis and objective to develop a novel, cost-effective, low-dose pharmacological intervention for empyema suitable for patients who are at elevated risk for surgery or conventional fibrinolytic therapy.

Keywords: empyema, therapy, fibrinolysis, targeting, mechanism, treatment

PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1).

A unique metastable protein, plasminogen activator inhibitor-1 (PAI-1)[†] [1] is a member of the serpin superfamily of proteinase inhibitors[2-6] which regulate normal and pathological thrombolysis and fibrinolysis. The conformational mechanism of PAI-1 (Figure 1) is driven by the negative free energy between the active (“stressed”) conformation with the Reactive Center Loop (RCL, Figure 1, pink) exposed to the solution and inactive (“relaxed”) conformations where the whole RCL or C-terminal part of cleaved RCL is inserted in the middle of the PAI-1 molecule as strand 4 of β -sheet A (Figure 1). Thus, active, stressed PAI-1 may be converted to a relaxed conformation with RCL inserted. Three major branches (Figure 1) follow this conformation: (i) spontaneous transition to an inactive latent form (k_{lat}); (ii) interrupting serine protease mechanism by stabilizing an acyl-enzyme intermediate (k_i); (iii) finishing

the protease mechanism resulting in a cleaved PAI-1 and active enzyme (k_s). Since $k_i \gg k_{lat}$ at physiological conditions, the inhibitory branch is >95% predominant if the target proteases, tPA and uPA, are present and inhibited by PAI-1 with the stoichiometry close to unity (Figure 1). Following RCL