Optimization of a Pulmonary Formulation of Tissue Plasminogen Activator for Pulmonary Delivery

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Objectives

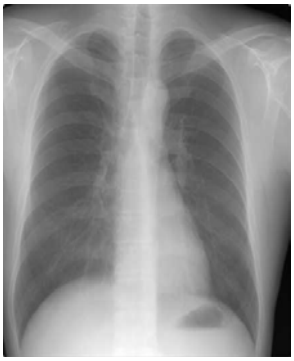
- Discuss the rationale for creating a pulmonary formulation of tPA (pf-tPA).
- Present data supporting the feasibility of nebulizing pf-tPA.
- Discuss the effect of nebulization on the self-association of pf-tPA.

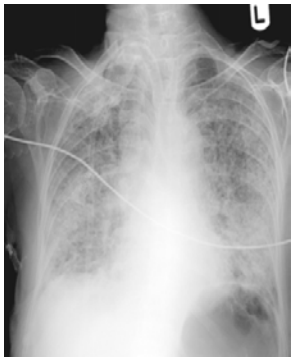
Tissue Plasminogen Activator

- Endogenous serine protease
- This protease is a significant contributor to the fibrinolytic pathway
  - Cleaves plasminogen to the fibrinolytically active form plasmin
- Primary clinical uses:
  - Dissolves thrombus associated with myocardial infarction and stroke
  - Also used to maintain catheter patency

Anti-Inflammatory Activity

- tPA also possesses anti-inflammatory activity that is:
  - Independent of fibrinolytic activity
  - Inhibits activator-induced oxidant production by neutrophils and macrophages
- tPA has been shown to inhibit inflammation in *in vivo* animal models
- This property of tPA could prove useful in the treatment of the Acute Respiratory Distress Syndrome (ARDS)





- ARDS is caused by damage to the alveolar epithelium and is characterized by:
  - Profound neutrophil infiltration into the lungs
  - Extensive inflammation and increased levels of proinflammatory cytokines (IL-1, TNFα)
  - Fibrin deposition which results in loss of lung compliance
- Currently there is no effective pharmacotherapy

(left) RadiologyInfo Website. http://www.radiologyinfo.org/content/chest_radiography.htm
(right) Evans, L. Norfolk and Norwich Hospital, Norwich, U.K. http://www.surgical-tutor.org.uk/default-home.htm?core/ITU/ards.htm~right

Need for a tPA Pulmonary Formulation

- IV administration of tPA for ARDS is not practical
  - IV administration results in disruption in coagulation homeostasis
- Pulmonary delivery would permit targeted delivery of tPA to the site of action
- Therefore, an optimal formulation was identified for pulmonary delivery
  - Protein must remain stable and active
  - Formulation must be safe and well tolerated

Protein Stability & Recovery

- Formulations were generated from Genentech's Activase ®
- Surfactant (Tween-80) was added to protein formulations in varying concentrations
- Stability and recovery was determined by UV absorbance at 280-400 nm
- tPA concentration range: 0.25-1 mg/mL
- Tween-80 surfactant concentration range: 0-0.5% (w/v)
- Prospective feasibility criteria were utilized to identify formulations feasible for nebulization.

Protein Stability & Recovery

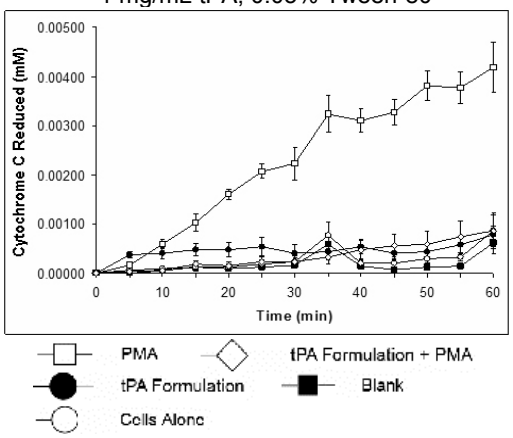
- 27 formulations were screened for protein stability and recovery parameters
  - UV spectra were obtained before and after nebulization
  - Permitted determination of protein loss or structural change caused by nebulization
- Feasible formulations exhibited high protein recovery and an aggregation index (AI) < 10
- 15 formulations met feasibility criteria and exhibited protein recovery ≥75%

Biologic Activity

- Anti-inflammatory activity of nebulized vs. non-nebulized tPA was assessed
  - Neutrophils were isolated from human peripheral blood for the assay
  - Cells were exposed to tPA formulation for 60 min
  - Neutrophil superoxide anion ($O_2^{\bullet-}$) production was induced by treatment of cells with phorbol myristate acetate (PMA)
  - $O_2^{\bullet-}$ production of the neutrophils was determined by the rate of cytochrome c reduction
  - Feasibility criteria required at least a 50% reduction in $O_2^{\bullet-}$ production

Anti-Inflammatory Activity

Formulation #1
1 mg/mL tPA; 0.05% Tween-80

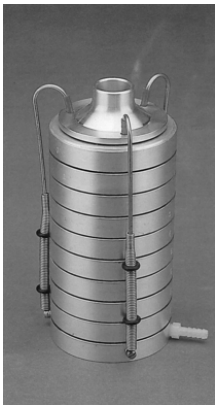


- Suppression (84%) of PMA-induced $O_2^{\bullet-}$ production
  - 1 mg/mL tPA; 0.05% Tween-80

Dunn, J.S., et al. *Pharm. Res.* (2005) 1700-7

Particle Size Determination

- Particle size of the aerosolized tPA formulations was determined by cascade impactor



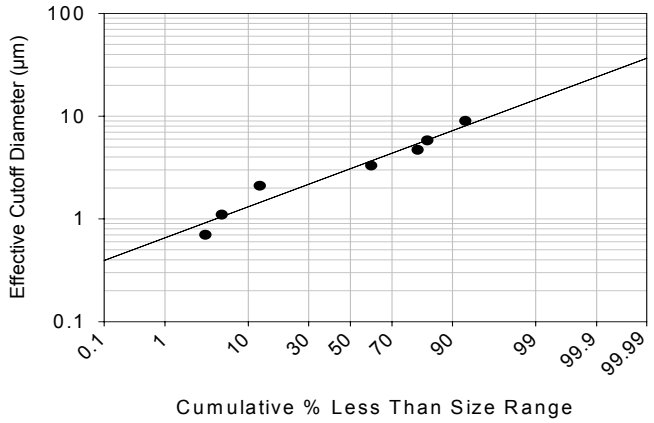
  - The cascade impactor captures ranges of particle sizes on plates located in each stage of the instrument
  - Cascade impactor data were represented on log-probability plots and linear regression was performed for further analysis

Particle Size Determination

- Parameters determined from cascade impactor data:
  - Mass Mean Diameter (MMD)
  - Geometric Standard Deviation (σ_g)
- Ideal parameters of an aerosol respirable to the lower airways
  - MMD ≤ 5 μm
  - σ_g > 1.2 μm
- Formulations exhibiting aerodynamic characteristics similar to these values were considered feasible

Particle Size Data

Formulation #1
1 mg/mL tPA; 0.05% Tween-80



- Formulation 1:
  - MMD = 2.4 µm (σ_g = 2.7 µm)

Dunn, J.S., et al. *Pharm. Res.* (2005) 1700-7

Conclusions

- 15 formulations remained stable following nebulization and exhibited >75% protein recovery
- pf-tPA formulation #1 reduced PMA-induced superoxide anion production from human neutrophils by >50%
- Formulation #1 exhibited optimal particle size distribution for aerosol pulmonary delivery

Optimization of Aerosol Collection

- Collection of pf-tPA induced ~25% insoluble aggregation
  - Efforts were made to optimize this collection method
- Nebulization-induced protein self-associations were quantitated by analytical ultracentrifugation (AUC)
- Biologic mechanism of pf-tPA anti-inflammatory activity is unknown and is being studied

pf-tPA Aerosol Collection

- Collection of pf-tPA induced ~25% insoluble aggregation
- Modified collection system permitted nebulization of protein, cooling in a condenser coil, and collection on silicon tubing
  - Insoluble aggregation was eliminated
- This method could be an effective means of collecting and analyzing aerosolized protein formulations

pf-tPA Aerosol Collection

Sample	Formulation	Post Nebulized Recovery	Aggregation Index (AI)	Percent Insoluble Aggregates
Flask Condenser System	1 mg/mL tPA; 0.05% Tween-80	0.71 mg/mL	2.8	23.5%
Condenser Coil System	1 mg/mL tPA; 0.05% Tween-80	1.27 mg/mL	4.4	None detectable

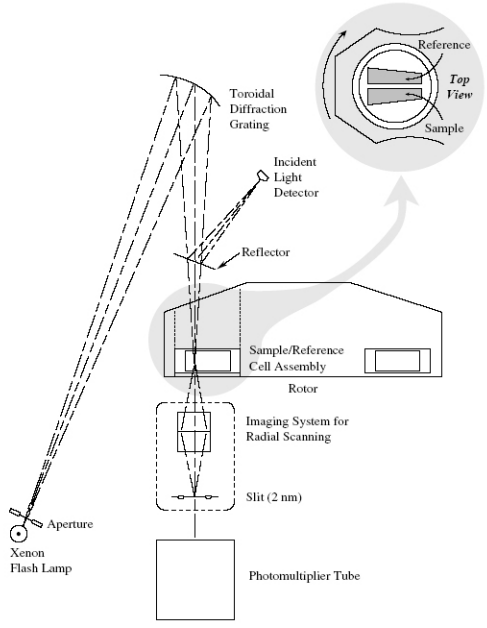
Self-Association Assessment

- Second derivative UV spectroscopy does not identify the nature of soluble aggregation
- Sought to determine the nature of soluble aggregates as a function of nebulization
  - Used AUC to accomplish this
  - Advantageous since the formulation can be assessed at 1 mg/mL in formulation buffer

Sedimentation Velocity of pf-tPA

- Prenebulized and nebulized pf-tPA was assessed by AUC
  - Three concentrations assessed (4, 8, and 16 μM)
- Sedimentation velocity was performed to assess self-association as a result of nebulization
  - Performed in a Beckman Coulter XL-A
  - Protein sedimented at 50,000 rpm, 4°C, 295nm
- Data fit performed for c(s) distribution with Sedfit 9.4 (Peter Schuck, NIH, http://www.analyticalultracentrifugation.com)

Analytical Ultracentrifuge Schematic



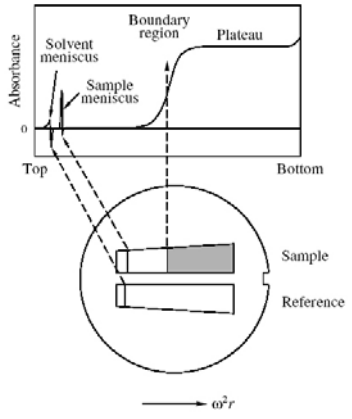
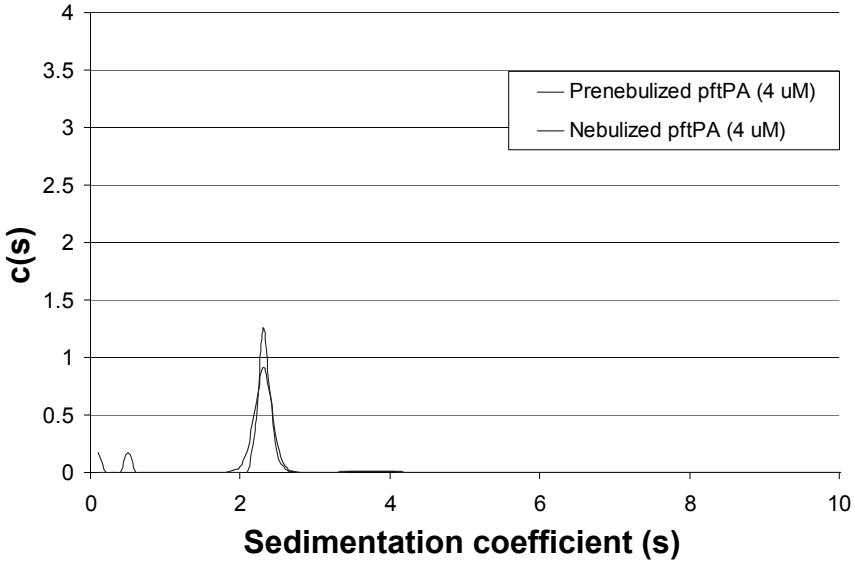


Image Reference:

Ralston, G. Introduction to Analytical Ultracentrifugation. Univ. of Sydney. Sydney, Australia

Sedimentation Velocity Results



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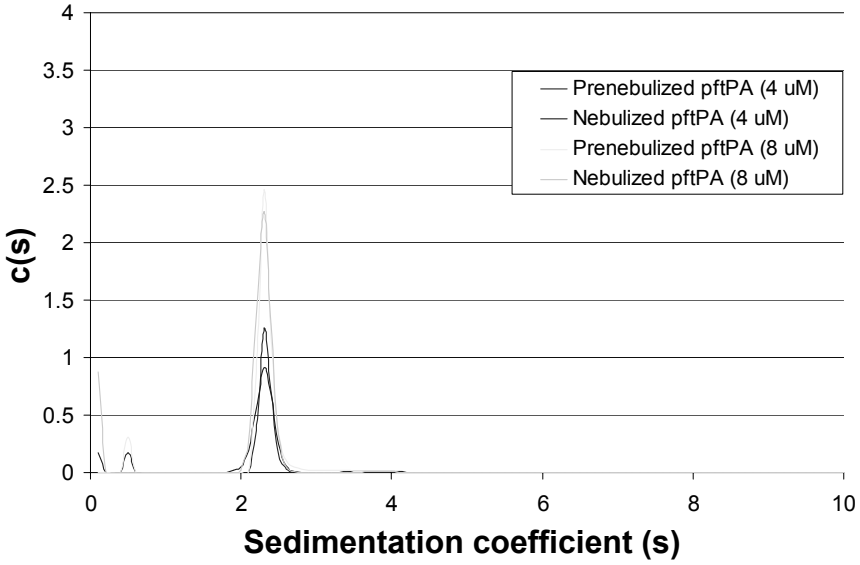
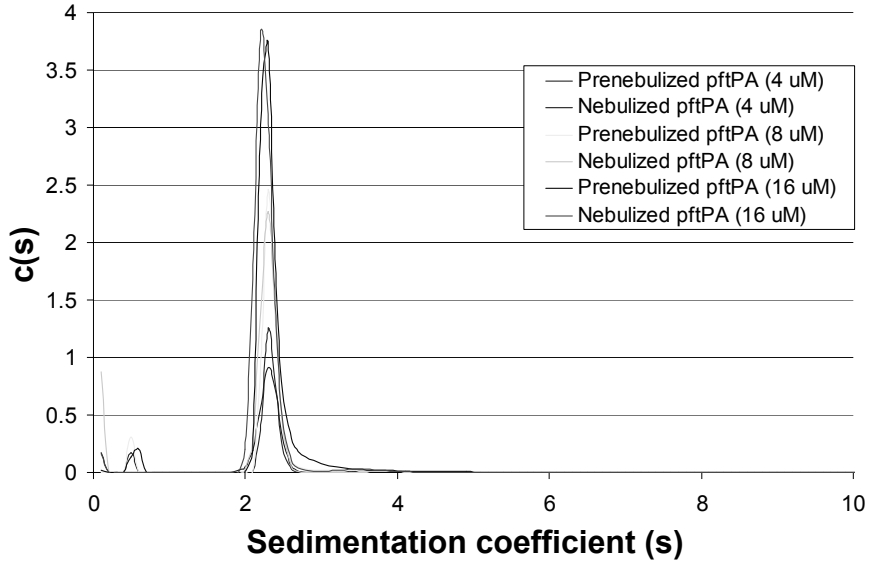


Sedimentation Velocity Results



Sedimentation Velocity Results

Sample	Sedimentation Coefficient (s)	Frictional Ratio (f/f_0)	RMSD
4 μM pf-tPA (Prenebulized)	2.34	1.41	4.87×10^{-3}
4 μM pf-tPA (Nebulized)	2.30	1.44	6.32×10^{-3}
8 μM pf-tPA (Prenebulized)	2.36	1.47	6.73×10^{-3}
8 μM pf-tPA (Nebulized)	2.30	1.34	7.43×10^{-3}
16 μM pf-tPA (Prenebulized)	2.33	1.41	8.59×10^{-3}
16 μM pf-tPA (Nebulized)	2.25	1.36	9.04×10^{-3}

Conclusions

- pf-tPA stability and recovery was improved by using a coil-condenser collection system
- pf-tPA remains monomeric following nebulization as determined by AUC
  - Nebulization of this protein formulation does not induce self-association
- Immunogenicity of pf-tPA will be determined by an *in vivo* study

Acknowledgments

- Kathleen A. Stringer, Pharm.D.
- Rajiv Nayar, Ph.D.
- David L. Bain, Ph.D.
- Keith Connaghan-Jones

- National Institutes of Health
  - HL071439-01A1
  - NIH Pharmaceutical Biotechnology Fellowship