Production and Preliminary *In vitro* Evaluation of a Plant-made, Oxidation Resistant Alpha-1 Antitrypsin

David Z. Silberstein[1], Kalimuthu Karuppanan[1], Hnin Hnin Aung[2], Ching-Hsien Chen[2], Carroll E. Cross[2], and Karen A. McDonald[1]

[1] Department of Chemical Engineering, University of California, Davis, CA

[2] Department of Internal Medicine

Alpha-1 antitrypsin deficiency (AATD) is the best-recognized genetic predispositioning factor to COPD in both smokers and non-smokers. Currently, the only specific treatment for AATD is intravenous replacement therapy with human alpha-1 antitrypsin (AAT) purified from pooled plasma, at a cost of over $100,000 per patient annually. Aerosolized AAT has shown potential for treatment of both AATD and cystic fibrosis; however, when inhaled into a neutrophilic inflammatory oxidative milieu, the oxidative susceptibility of methionine residues at positions 351 and 358 in the reactive site loop potentially leads to oxidative inactivation. Using a novel cucumber mosaic virus-based inducible transient expression system, we have produced a biobetter plant recombinant AAT (prAAT) in *Nicotiana benthamiana*. This variant replaces the oxidation-susceptible methionine residue at position 358 with a valine residue to increase resistance to oxidation.

N. benthamiana plants were vacuum infiltrated as detached leaves with two strains of *Agrobacterium tumefaciens*: one carrying the biobetter AAT gene and one carrying a gene for the tomato bushy stunt virus p19 viral RNA silencing suppressor driven by a constitutive cauliflower mosaic virus 35S promoter. Leaves were incubated in humidity chambers at 20 °C in the dark for 6 days before flash freezing in liquid nitrogen, grinding, and protein extraction in a 20 mM Tris, 150 mM NaCl, and 0.01% (v/v) Tween-80 AAT stability buffer. Purification was achieved using Alpha-1 Antitrypsin Select Affinity Chromatography (GE Healthcare) followed by concentration and diafiltration using Amicon 30 kDa NMWCO spin columns with a 58±8% yield of active AAT.

Purified prAAT was tested against an analytical standard (Calbiochem) and Prolastin-C (Griffols), a common therapeutic formulation of human AAT, to determine its activity and oxidation resistance. 41±11% of purified prAAT and 71.9±5.5% of Prolastin-C showed activity against porcine pancreatic elastase as determined by a residual elastase activity inhibitory assay. Under oxidation with 48.9mM H_2O_2 for 60 minutes at room temperature, prAAT was found to retain 102.8±20.4% of its original anti-elastase activity, while Prolastin-C retained 13.8±5.0% anti-elastase activity. Under oxidation with 100nM NaOCl for 15 minutes at room temperature, prAAT was found to retain 99.9±9.7% of its original anti-elastase activity, while Prolastin-C retained 34.2±24.1% anti-elastase activity. Human bronchial epithelial cells were grown in a 96 well plate and exposed to human neutrophil elastase and either prAAT or Prolastin-C. After 48 hours of incubation post-exposure, both prAAT and Prolastin-C showed similar protection against elastase-induced cell death.

We are currently investigating our prAAT's resistance to oxidation by hypochlorite. Future work will include optimization of downstream processing and further preclinical studies of prAAT toxicity and efficacy.