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Disposition of Alpha-1-Antitrypsin in the Isolated Perfused Rabbit Lung

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ABSTRACT

The potential for delivering large molecular weight proteins into the lungs to reach local or systemic sites of action was investigated by examining the disposition of alpha-1-antitrypsin in the isolated rabbit lung. Alpha-1-antitrypsin, a model protein, was measured in the perfusion medium following intravascular administration and was found to remain constant, indicating limited uptake or metabolism by lung tissue. Intrabronchial instillation of 10 mg of alpha-1-antitrypsin in water resulted in no measurable concentration in the recirculating perfusate during the two hours experiment. These data suggest that transport of large proteins may be limited across lung-blood membrane barriers in either direction. Though this would limit the ability of inhaled drugs with large molecular weights to reach the general circulation, proteins which are used to treat respiratory diseases, such as alpha-1-antitrypsin, might be delivered locally by inhalation with only negligible systemic exposure.

Key words: Alpha-1-Antitrypsin, Alpha-1-Proteinase Inhibitor, Protein, Pulmonary absorption, Isolated perfused rabbit lung

INTRODUCTION

Many non-oral pathways or drug delivery systems for the administration of proteins and peptides have been explored to circumvent their poor absorption and providing systemic availability as well as local site specific activity (1, 2). Pulmonary drug delivery provides therapeutic benefits for a wide range of protein-based drugs, especially for biotechnology products which many problems for their administrations exist. The possibility that an inhaled protein, peptide or any compound may be absorbed through the lungs depends on many factors (3). Many substances are absorbed by the lung and produce systemic pharmacologic or toxicologic effects, furnishing evidence for a pulmonary route of administration for drugs to reach sites of action apart from the pulmonary tract. Investigation of the pulmonary disposition of proteins may also define the potential for delivering these large molecular weight entities to the general circulation by inhalation. On the other hand, if there is limited absorption by the pulmonary route, the opportunity still exists for the delivery of proteins necessary to provide a local therapeutic effect within lung tissue, without systemic exposure to the biomolecule. However if drug absorption from the respiratory tract is slow, delivery of a protein to a site of action from lungs would provide an optimal method of drug administration. The objective of this investigation was to examine the pulmonary absorption and disposition of a model protein, Alpha-1-antitrypsin (AAT) is a large molecular weight (54,000 daltons) protease inhibitor. This glycoprotein is synthesized in the liver, has normal serum concentrations of 1.8-2.8 mg/ml and a normal serum half-life of five to seven days (4). Deficiency of AAT (also called Alpha-1-protease inhibitor) disrupts the

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protease-anti-protease balance within the lung and results in dyspnea, emphysema, or other pulmonary difficulties An injectable formulation of this protein has been approved as a replacement in those patients who are genetically deficient in AAT production (5). Also astudy assessing the aerosol delivery of AAT has been reported (6). Hence the distribution of AAT from the blood into the lung and the retention of AAT in lung tissue after pulmonary administration are of importance to be determined.

MATERIALS AND METHODS

Isolated perfused rabbit lung:

These studies were performed in an isolated perfused rabbit lung system similar to that described previously by Mayer et al. (7). New Zealand white albino male rabbits (2- 4 Kg) were anesthetized with 50 mg/kg pentobarbital and were treated with 1000 units/kg heparin. Cardiac puncture was performed and the trachea, lungs and heart were removed. After removal of extraneous tissue, the pulmonary artery, trachea and left atrium were cannulated and the organs were suspended in the lung chamber(Figure.1). A volume of 100 ml of the perfusion medium (Krebs and Henseleit, 1932) containing 4.5% bovine serum albumin and 1mM glucose was allowed to re-circulate through the lung at the rate of 150 ml/minute at 37°C and pH 7.40.

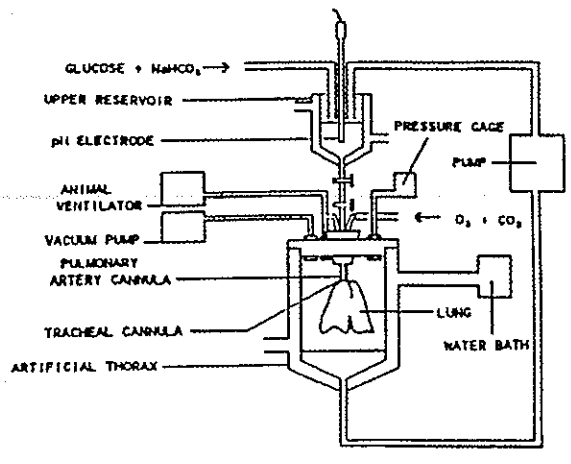

Fig1. Schematic representation of the isolated perfused rabbit lung.

A warmed, humidified air-carbon dioxide ventilating mixture was supplied to the lungs, which were respiring at 50 cycles per minute. The intravascular experiments were performed by adding 10 mg of AAT directly to the perfusion medium in the upper reservoir (n= 4). A Control experiment (n=3) utilizing a solution of AAT recirculating through apparatus in the absence of a rabbit lung was also performed. For intrabronchial administration (n=6), the drug was instilled into the bronchioles via polyethylene tubing inserted through the tracheal cannula into the airways (8). Dosing solutions was 250 microlitre containing 10 mg of ATT (Sigma, St. Louis, MO) in distilled water. One mililitre of the perfusion medium samples were withdrawn from the upper reservoir after the lung had equilibrated prior to dosing and at 5,15, 30, 60 and 120 minutes after dosing. The samples were frozen immediately and kept at -20°C until assay. Following the conclusion of each experiment the lung was homogenized in two fold normal saline, centrifuged at 10,000 g, and aliquot of the supernatant was retained for analysis.

Analytical Method:

The perfusion medium samples were assayed for AAT concentration by a radial immuno-diffusion (RID) technique (9), according to the procedure provided by the manufacturer (Calbiochem- Behring, La Jolla, CA). Twelve well RID plates were utilized and 20 microlitre aliquots of standard solutions or samples were dispensed into the wells. The plates were incubated for three days at room temperature and the diameter of the precipitin ring was measured with a calibrated eyepiece. A five point calibration curve was obtained by plotting the squares of the ring diameters versus AAT concentration from 30-120 µg/ml. The AAT concentrations were determined for each sample from the least squares estimate of the calibration curve.

RESULTS AND DISCUSSION

Analysis of the perfusion medium samples obtained from control experiments and four intravascular AAT experiments indicate no

change in concentration during the 120 minute perfusion of the lung (Figure 2). These findings suggest that there is no or very limited metabolism of AAT by the lung and only a slight distribution of AAT, into the lung from the vasculature. This negligible uptake of AAT into pulmonary tissue was also confirmed by assaying lung homogenate for AAT. In only one experiment measurable amounts of AAT were recovered from the lung tissue. In this instance, 1.2 mg of AAT (12% of the dose) were present in the lung at the end of two hours. The average of the total recovery of AAT from these experiments were greater than 90% of the administered dose. After intrabronchial administration of AAT to the isolated perfused rabbit lung, no measurable AAT could be detected in the perfusion medium. A lack of sufficient assay sensitivity (30 μg/ml) contributed to the problem of ascertaining whether AAT was able to be absorbed through the lung parenchyma. However, radial immunodiffusion still remains the assay of choice for endogenous AAT. It is most probable that AAT was absorbed but was not detected in the perfusion medium because only an average of 60% of the instilled dose could be found in the homogenized lung tissue for 120 minutes after dosing. If the perfusion concentration of AAT approached the assay sensitivity, but were still undetectable, the remainder of the administered dose would account the maintenance of the mass balance. A control experiment was performed to demonstrate the complete recovery of the drug from lung tissue; 104.5% of the administered dose was recovered from lung homogenate three minutes after dosing. Some individuals are genetically deficient in AAT due to the inheritance of a defective structural gene(10).

Therefore, a general therapeutic approach for patients severely deficient in AAT provides replacement therapy (11). Intravenous infusion of 60mg/kg of AAT on a weekly basis raised serum AAT levels four fold in an investigation designed to evaluate the safety and efficacy of the parenteral AAT infusions (12).This approach would raise plasma concentrations of AAT, but would necessitate distribution of AAT into bronchoalveolar lavage fluid. Our data suggest, only slight amount of AAT may diffuse or be transported into lung tissue, though it is possible that therapeutic amounts of the protein may reach appropriate sites of activity. In a study of the pulmonary penetration of AAT after parenteral administration in dogs it was found that due to the 103 hour half - life of AAT in blood, AAT concentrations in lavage fluid increased 24 hours in spite of a lack of preferential uptake by the lung (13).

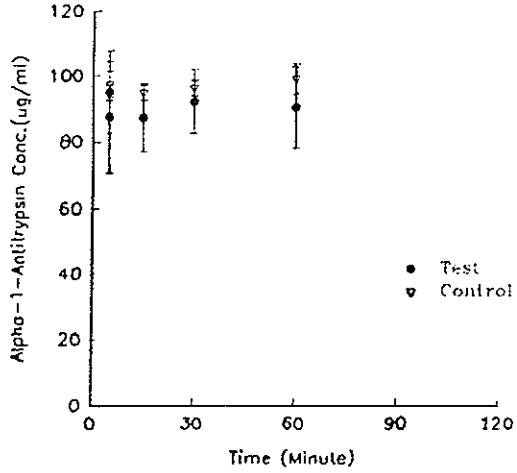

Fig. 2 Alpha-1-antitrypsin perfusion medium concentration versus time profile following 10 mg intravascular administration to isolated perfused rabbit lung (n=3-4). Error bars represent one standard deviation.

Therefore, AAT dose not appear to concentrate in the lung and lung AAT concentrations do not parallel plasma AAT concentrations. Limited distribution of ^{125}I-AAT into rabbit lungs has been observed up to one week following a single intravenous injection, while significant AAT activity was found in the bronchoalveolar lavage fluid of monkeys six hours after administration of the intravenous AAT (14). The hypothesis of the relation between the permeability of the lung to proteins and molecular weight has been investigated previously (15). It is suggested that proteins smaller than AAT would have greater access to the lung if present in the general circulation, while proteins of much

higher molecular weight would have more restricted access to the lung. The retention of AAT in the lung would promote its pharmacologic activity in this tar-get organ. It has previously been suggested that aerosol delivery of AAT to the lung may provide more efficient delivery of this antiprotease than the intravenous route (13). Preliminary evidence has indicated that this type of protein delivery provides AAT in the lung epithelial lining fluid and lung lymph (16,17). A report on the aerosolized administration of ^{131}I-AAT into the lungs of dogs indicated that the homogenous distribution of AAT was observed with large amounts of AAT present in bronchoalveolar lavage fluid six hours after administration (6). This finding agrees with the data presented in this study, where significant amounts of AAT were retained in lung tissue following intratracheal instillation.

CONCLUSION

The results of this study demonstrates that the permeability of AAT across the lung/blood interface is limited in both directions when AAT is present in the general circulation or in lung tissue by virtue of adminstration through the pulmonary air-ways. Thus, it is more likely that a protein of large molecular weight, such as AAT, would be suitable to provide local pharmacologic activity directly in the lung following pulmonary delivery. This mode of drug delivery would be appropriate for AAT replacement therapy in patients deficient of this protease inhibitor. Systematic investigation of the pulmonary absorption of various molecular weight proteins should provide evidence for the feasibility of delivering other proteins by this route.

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