Delivery of low molecular weight antioxidants into rat lungs post exposure to Cl_2 gas

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INTRODUCTION

Chlorine (Cl_2) is a moderately soluble, highly reactive oxidant gas, used extensively for water purification, manufacturing of pharmaceuticals and chemicals and as a potent disinfectant. More than 25 million tons of chlorine is manufactured annually in the United States alone and the majority of this gas is transported by rail. Large quantities of chlorine gas have been released into the atmosphere following industrial accidents in production facilities or during transportation of chlorine to end users. Our data so far shows that exposure of rats to Cl_2 to either 187 or 400 ppm for 30 min severely decreases ascorbate levels in BAL and lung tissues and GSH/GSSG ratios in lung tissues. Furthermore, prophylactic intramuscular and intravenous injection of ascorbate, NAC and desferal increases ascorbate levels in BAL, normalizes GSH/GSSG values in lung tissue and decreases the extent of Cl_2 induced injury to the alveolar epithelium (in review).

In this series of experiments, we developed an aerosol system to deliver aerosols capable of reaching the distal lung epithelial surfaces. We then used this system to delivere aerosolized ascorbate and deferoxamine into the lungs of mice, post Cl_2 exposure. Our results indicate that we can replete depleted ascorbate levels without damaging the alveolar epithelium.

PURPOSE

- Expose rats to Cl_2 (300 ppm for 30 min) in an environmental exposure chamber, and return to room air.
- One hour after exposure, deliver aeroslolized ascorbate and deferoxamine via aerosol for one hour. Control animals received vehicle.
- Sacrifice the rats; perform BAL and remove lung tissues; obtain blood sample.
- Measure levels of ascorbate, reduced (GSH) and oxidized glutathione (GSSG) and urea concentrations in BAL, plasma and lung tissues.
- Calculate the volume of epithelial lining fluid and amounts of AA, GSH and GSSG in ELF and lung tissue.

RELEVANCE TO THE MISSION OF THE CounterACT PROGRAM

- Chlorine gas was among the first chemical weapons to be used in modern warfare. During the last year Cl_2 cylinders were packaged with explosive materials by insurgents in at least four incidents in the Iraq war. In addition, nine people were killed and 250 injured in 2005 after a train crash in South Carolina in which 60 tons of liquefied chlorine was released to the atmosphere.
- Our previous findings suggest that prophylactic administration of ascorbate (Vitamin C) and deferoxamine (an iron chelator used to remove excess iron from the body) significantly reduce Cl_2 injury to the lung and restore arterial oxygenation to nearly normal levels.
- Herein, we compared the efficacies of various modes of administration of ascorbate and desferoxamine into the lungs of rats post Cl_2 exposure. The aim of these studies is to develop the most efficient and least invasive delivery mode of replenishing depleted antioxidant defenses in civilian and military personnel exposed to Cl_2.

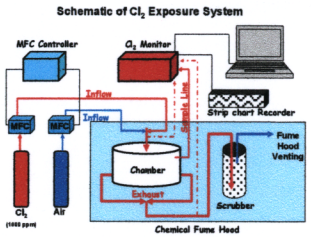


Figure 1. Schematic diagram showing important features of the Cl_2 exposure chamber. Air and Cl_2 mix before entering the chamber; further mixing is achieved inside the chamber while they pass through a diffuser. Total gas flow rate in the chamber is 5 L/min which allows for about 1 full exchange of the chamber atmosphere per min.

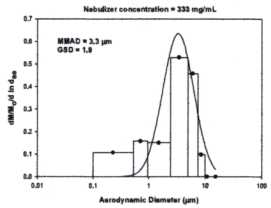
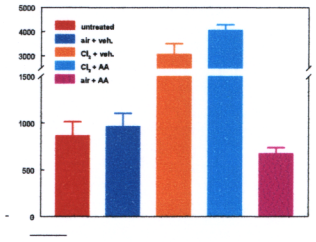
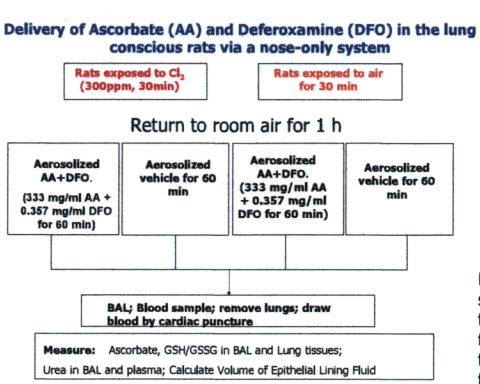


Figure 3. Representative particle size distribution of anti-oxidant aerosols determined for the indicated nebulizer concentration. Particle size distribution is presented as Mass Median Aerodynamic Diameter (MMAD), mm and Geometric Standard Deviation (GSD). $dM/Mo/d\ ln(d_{ae})$ is the cumulative mass fraction of particles collected on each stage as a function of particle diameter.

Figure 4. Total protein in ELF (μgr)





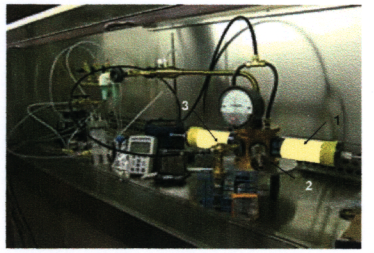


Figure 2 : The aerosol generating and delivery system inside a Biosafety II hood. Arrows point to the components listed below: **1.** Nose only restraint tube for rats. **2.** Cascade impactor for the analysis of the aerodynamic particle size distribution; **3.** 25 mm fillter holders for gravimetric determination of the aerosol concentration.

Figure 5. Epithelial Lining Vol. (μl)

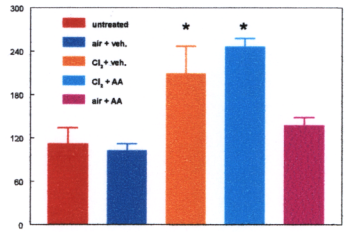


Figure 6. Ascorbic Acid in Lung (nmoles/lung)

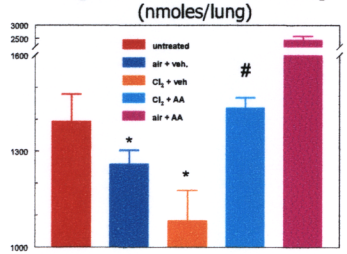


Figure 7. Ascorbic Acid in ELF (nmoles)

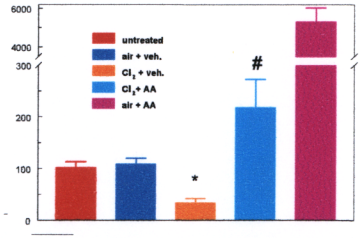
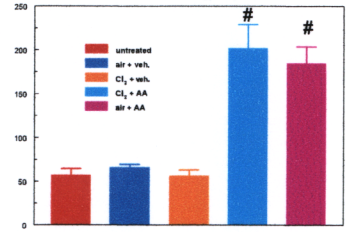


Figure 8. Ascorbic Acid in Plasma (μM)



*, # p<0.05 compared to untreated and Cl2 vehicle, respectively

Conclusions

Aerosol delivery of ascorbate in conscious rats post Cl_2 exposure restores depleted ascorbate levels in epithelial lining fluid and lung tissues without damaging the lungs.

Future Directions

We are currently evaluating whether restoration of depleted AA reverses Cl_2 injury to the alveolar epithelium. We also plan to use this system to deliver combinations of protective agents such as β2-agonists, nitrite and low molecular weight scavengers.