

Respiratory System Deposition with a Novel Aerosol Delivery System in Spontaneously Breathing Healthy Adults

Running Title: Respiratory System Deposition Using a Novel Aerosol Delivery System

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Summary Background: Intravenous magnesium sulphate (MgSO_4) in children and adults with refractory acute asthma is effective but therapy may be limited by systemic hypotension which might be avoided with the aerosol route. Inhaled MgSO_4 has a relatively high dose (volume) requirement. This, plus the use of inefficient delivery systems, may explain the lack of efficacy of inhaled MgSO_4 in some studies. An *in vitro* study suggested that the AeroNeb Go[®] with the Idehaler Pocket[®] using a face mask would deliver 16 mg/min of MgSO_4 to the respiratory system for older children and approximately a fifth for toddlers but no *in vivo* data exist. Materials and Methods: Saline mixed with a radiolabel was used as a proxy for the 100 mg/mL MgSO_4 solution. In 5 adult males the rate of deposition was measured using nuclear medicine techniques. The deposition below the vocal cords of the radiolabel was converted to the rate of deposition of MgSO_4 and was compared to the results from an *in vitro* model using adult respiratory patterns. Results: The mean rate of deposition was 12.6 ± 1.9 mg/min (mean \pm SD). The reasons for this lower deposition compared to the *in vitro* estimate was likely exhalation of anatomical dead space aerosol which would have been captured on the inspiratory filter *in vitro*. Conclusions: These *in vivo* data confirm the deposition data predicted in the *in vitro* study although caution should be used extrapolating to children. This device appears suitable for the clinical trial of inhaled MgSO_4 in children and adults with refractory asthma.

Key words: asthma, inhaled magnesium sulphate, Idehaler Pocket[®], AeroNeb Go[®], nuclear medicine, respiratory system deposition, pulmonary deposition

Introduction

A poor response to inhaled beta-2 agonists[1] and delayed response to corticosteroids in a subset of children with acute asthma has brought about interest in the use of magnesium sulphate (MgSO_4). Two recent meta-analyses[2;3] suggest a reduction in hospitalization with intravenous (IV) MgSO_4 . However, this requires the use of an IV route and is associated with a risk of systemic hypotension which represents a major barrier to using IV MgSO_4 in North America [4]. On the other hand, the aerosolized route of delivery is non-invasive and direct way to deliver a high concentration of MgSO_4 to the airway epithelium and the smooth muscle below without depending on delivery via a high blood level and its associated potential for toxicity[5]. Although the preliminary evidence suggests clear additive benefit of MgSO_4 and albuterol on lung function in adults with severe disease and a trend towards benefit with respect to lung function and hospitalizations in moderate asthma[2;6], the efficacy of nebulized MgSO_4 in children is unknown. A major limitation of all studies to date, both in adults[7] and children[2] was the use of inefficient aerosol delivery methods. Given the preliminary evidence of benefit, the non-invasiveness and high safety likelihood of the nebulization route, a pediatric study is needed to define the role of nebulized MgSO_4 [2;6;8]. Most aerosolized drugs such as albuterol are efficacious in the microgram range, whereas MgSO_4 is only efficacious in the milligram range and hence requires many-fold higher drug delivery with a physical limitation of the nebulizable volume rate of output. Increasing the MgSO_4 concentration would increase the rate of delivery but this could only be achieved with aerosol tonicity of greater than 500 mOsm/L which has been associated with bronchospasm[9].

In preparation for a large multicenter study to test the benefit of inhaled MgSO_4 , *in vitro* studies were done to select an appropriate delivery system [10]. Due to both the wide age range

of the proposed subjects and the desire to use a single aerosol delivery system, a face mask rather than a mouth piece was required which precluded the use of high efficiency breath enhanced or breath actuated nebulizers since they require one way valves and a tight fit with the airway opening. Using breathing patterns ranging from that of adults to small children, *in vitro* studies suggested that the AeroNeb Go[®] (Aerogen, Galway, Ireland) vibrating mesh nebulizer coupled with the novel holding chamber the Idehaler Pocket[®] (La Diffusion Technique Francaise, St Etienne, France) (Figure 1) with a charge volume of 6 mL would deliver approximately 16 mg/min of a 100 mg/mL solution of MgSO₄ in older children and adults and roughly a fifth of this when using a breathing pattern suitable for toddlers[10]. In that study, the *in vitro* respirable fraction (RF) was defined as the mass of aerosol carried in particles $\leq 5 \mu\text{m}$ with the expectation that particles in this size range would, if inhaled, deposit below the vocal cords. Hence, respiratory system deposition was estimated by the multiplying the amount of aerosol collected at the filter at the “mouth” by the RF.

The purpose of this pilot study was to confirm the *in vitro* estimates in normal adult males using nuclear medicine techniques that account for clearance of the aerosol during both the delivery period and any delay between the end of nebulization and imaging[11]. Confirmation of accuracy for the *in vitro* data for adults would validate both the performance of this novel delivery system and the *in vitro* methodology used in the estimates of respiratory system deposition. It is recognized that, ideally, children with acute asthma should be part of the study but this was not considered acceptable for ethical reasons.

Materials and Methods

Five healthy non smoking males between 40 and 67 years of age who were familiar with the protocol, risks and methods participated in the study. Males were chosen to avoid problems with different anterior and posterior chest wall attenuations due to breast tissue [12]. All participants signed informed consent and the protocol had been approved by the institution's research ethics board. Since there was no reason to suspect that saline would deposit any differently than the aqueous solution MgSO_4 , normal saline was used in its place in order to eliminate any potential pharmacological effect associated with the use of MgSO_4 . Based on *in vitro* data [10], approximately 100 MBq of technetium attached to diethylenetriaminepentaacetic acid ($^{99\text{m}}\text{Tc-DTPA}$) was added to 6 mL of normal saline and placed in the reservoir of the AeroNeb Go[®] coupled to holding chamber, the Idehaler Pocket[®] with a face mask as the patient interface (Figure 1). The nebulizer generates a constant flow of aerosol through the membrane as long as there is fluid on the reservoir side. When there is none, it stops. In order to avoid saturation of the camera from the point source of radiation [12], the charged nebulizer was counted on 12 Lucite[®] plates 0.5 cm thick; each with a known attenuation between the nebulizer and the camera[12]. After acquisition, the counts received by the camera can be “scaled up” (e.g. counts divided by 0.4) to what they would have been without the Lucite[®] plates. The vibrating mesh of the nebulizer is metal and also attenuates the signal but if the nebulizer is inverted, the thin plastic top has no measurable attenuation (eg counts in the syringe before loading the nebulizer are the same as counts in the inverted nebulizer after loading). Prior to the inhalation, tissue attenuation was measured using the Marshal and Macey[13] technique with a cobalt flood source.

In the aerosol laboratory, the subjects inhaled from the device until dryness which was approximately 12 minutes. Their upper body was in negative pressure hood evacuated to the

outside of the aerosol laboratory to remove any free radioactive aerosol. Following the inhalation, they gargled and then drank a glass of water in order to wash oral and esophageal radiolabel into the stomach. The inhalation was done while seated but simultaneous anterior and posterior imaging was performed supine to avoid motion artifact. Post inhalation, the subjects went to the Nuclear Medicine facility and were scanned for one minute at five minute intervals between the two heads of the gamma camera (GE Millennium MG, Milwaukee, Ws) in order to determine the total dose deposited in their lungs, and clearance of the radiolabel from their lungs. Both heads were tuned using a symmetrical 10% window. Counting was done as soon as practically possible after the end of nebulization, usually between 5 and 8 minutes, and then 5, 10, 15 and 20 minutes later. Details of this technique have been described previously[11] with the exception that it was impossible to compare the amount of radioactivity pre nebulization to post since the absence of an “expiratory filter” prevented capturing all aerosol as a measure of quality control.

Calculations

In order to minimize radiation exposure, a pre inhalation xenon scan was not done and the Regions of Interest (ROIs) were obtained from the first image where the lung has the highest number of counts and offers the clearest definition of the lung borders[14] (Figure 2). Details of the selection of the ROIs for the stomach, lungs and mediastinum have been well described recently in healthy subjects [11]. All counts were corrected for radioactive decay based on a half life of 6 hours starting at the time of imaging of the charged nebulizer pre nebulization. During the administration of the aerosol, the ^{99}Tc -DTPA is being constantly removed by absorption into the pulmonary circulation and by mucociliary clearance. Since imaging was carried out at least 15 minutes after the start of nebulization, the counts in the lung regions at the

first acquisition needed to be adjusted for subsequent decline within this period. The natural logarithm of counts per pixel for the lung ROIs was plotted against the time of acquisition from the 1st to the 5th acquisition occurring 20 minutes later [11]. It is assumed that the radiolabel is deposited at a constant rate [15] and that its clearance is a time dependent exponential curve. A linear regression was done using the natural logarithm counts versus time and the inverse of the slope is the time constant (τ) of the lung clearance. Linearity of the logarithmic transformed data was assured if the Pearson correlation coefficient was > 0.96 (which it always was). The total time of nebulization (t_f) was then broken down into 5 epochs with the center of each epoch being t_1, t_2 to t_5 . Hence, for each increment of time, 20% of the total deposition occurred and part was removed by the lung clearance mechanisms according to the time constant τ . Hence, the amount in the lung at the end of nebulization was:

$$\Sigma[(0.2 e^{-(t_f-t_1)/\tau}) + 0.2 e^{-(t_f-t_2)/\tau} + 0.2 e^{-(t_f-t_3)/\tau} + 0.2 e^{-(t_f-t_4)/\tau} + 0.2 e^{-(t_f-t_5)/\tau}]$$

where Σ is the sum and “e” the base of the natural logarithm. There is a delay (t_d) between the end of nebulization and the time it takes to move the subject from the aerosol laboratory to gamma camera and begin counting. Hence the entire expression above was multiplied by $e^{(-t_d/\tau)}$.

The resulting value then represents the fraction of the radiation that was deposited in the lung and remained in the lungs at the time of counting. The inverse of this fraction multiplied by the total counts in the first image represented the total pulmonary deposition. Greater details of these calculations have been published recently in both healthy adults [11] and children and adults with lung disease[16].

If MgSO_4 had been used instead of saline, the total amount of counts in the nebulizer prior to nebulization would represent 600 mg of MgSO_4 in 6 mL of a 100 mg/mL solution.

Hence, the total counts in the nebulizer prior to nebulization, divided by 600 mg gives the counts

per mg of MgSO_4 . Hence, the total counts in the lungs and trachea, corrected for all the factors listed above, divided by the total counts in the nebulizer pre nebulization and multiplied by 600 is the respiratory system deposition expressed as mg of MgSO_4 . Dividing by the nebulization time, the rate of deposition is expressed as mg/min of MgSO_4 .

Results

The subjects ranged in height from 173 to 188 cm and in weight from 62 to 92 kg (Table). The mean rate of deposition was 12.6 ± 1.9 mg/min [mean \pm standard deviation (SD)] (95% CI 10.9, 14.2) and a range of 11.2 to 15.9 mg/min (Table). Due to both the time for nebulization and the delay in reaching Nuclear Medicine, the correction factor for clearance of the radiolabel was 0.87 ± 0.04 (mean \pm SD) indicating that 13% of the lung deposition had been cleared by the time of the first image. The *in vivo* RF, defined as the lung deposition divided by the total body deposition, was 0.57 ± 0.05 (mean \pm SD). Looked at over the course of approximately 12 minutes of nebulization, roughly 25% of the charge dose would be deposited in the lungs. The nebulizations were well tolerated with no cough or discomfort. Expressing the data in the *in vitro* study[10] as the mean and 95% confidence intervals, the 16.0 (15.6,16.5) mg/min does not overlaps the 12.6 (10.9,14.2) mg/min of the *in vivo* study implying differences that are significant.

Discussion

This novel nebulizer and holding chamber combination, while not as efficient as modern nebulizing systems[17] such as the PARI eFlow[®] with one way valves and a tight seal at

the airway opening, is certainly as good or better than the breath enhanced nebulizer PARI LC PLUS[®] used for delivering tobramycin[18] where roughly 15% of the 300 mg charge dose in a 5 mL volume deposited in the lungs in a study including both adults and children and roughly the same as a 4 mL charge delivered by the PARI LC STAR[®] in adults[16]. Importantly, this device is orders of magnitude more efficient than unvented jet nebulizers with a face mask[19;20]. Furthermore while, Chua and colleagues[19] showed extremely low deposition in the order of 1% of the emitted nebulizer dose in infants, they found that roughly 4% of the emitted dose deposited in the lungs of older children breathing from a face mask, whereas the novel system in the present study found roughly 25% of the charge dose deposited in the respiratory system. Hence one would anticipate up to a five fold increase if one can extrapolate the difference between their system in older children and the current one used in adults. The *in vivo* RF of 0.57 ± 0.05 agreed with that measured by laser diffraction *in vitro* of 0.59 ± 0.0 [10] which corresponded to a mass median diameter of $4.4 \pm 0.1 \mu\text{m}$, assuming unit density.. The *in vitro* RF depends only on measured particle size distribution[21] and is based on the probability that this fraction of the mass of the particles within a certain size range will, if entering the upper airway, deposit below the vocal cords. Bases on previous literature[22-24], the *in vitro* size cut off was $\leq 5 \mu\text{m}$ and the *in vivo* agreement is support for this choice. *A priori* it was recognized that *in vivo* measurements would be less than *in vitro* because during the last phase of inspiration aerosol enters the anatomical dead space, predominately the trachea and some of which remains in suspension is exhaled at the start of expiration[25;26]. *In vivo*, this does not appear as deposition but *in vitro*, it is captured on a filter at the “airway opening” of the mechanical breath simulator during *in vitro* studies. During quiet breathing, anatomical dead space represents roughly of 25% of the tidal volume[27;28] and includes the entire trachea but tracheal images do indicate

deposition so, while some particles may have been exhaled, others remained. Despite attempts to wash all the radiolabel out of the upper airway and esophagus, there was a small amount of residual left in the esophagus distal to the bifurcation of the trachea so it is recognized that including the mediastinum in the deposition below the cords will lead to a slight overestimation of both deposition and the *in vivo* RF. However, Figure 2 would suggest that esophageal deposition was minimal. It is also recognized that tracheal and main stem bronchi deposition will likely have little therapeutic effect on bronchoconstricted airways.

If a constant rate of pulmonary deposition, analogous to an intravenous infusion, is being sought, there are problems with jet nebulizers. First, evaporative losses result in a progressive concentration of drug in the well of the nebulizer[15] and hence an increasing rate of aerosol production if measured in quantity of drug per minute over the course of nebulization and increasing osmolarity of the aerosol droplets. Second, towards the end of nebulization sputtering occurs and the production progressively falls[18]. On the other hand, mesh nebulizers extrude the drug solution through microscopic pores at a constant rate with minimal chance for evaporation in the small holding chamber so the rate of lung deposition and osmolarity should be constant providing the pattern of ventilation is constant[11]. Furthermore, most vibrating mesh systems have a controller that indicates when nebulization has ceased allowing the session to be terminated or alternatively, the nebulizer refilled. This is very similar to a constant intravenous infusion where modern infusion pumps deliver drugs at a constant rate and indicate when the infusion has been completed.

Compared to other studies where the internal control for accuracy was the agreement of the amount of radioactivity pre versus post-nebulization [11;12], this study lacks such a control. Since the goal of the study was to test a system with a face mask but without valves, a filter on

the expiratory limb was not practical so aerosol generated that did not deposit in the body was lost to the environment. A second limitation is a concern to what degree the results in normal adult males would be generalizable to children with acute asthma. While a study on such population may have been ideal, there were serious ethical concerns about having sick children enrolled in a research study in an area not designed for patients with acute respiratory distress. While saline was used rather than MgSO_4 , there is no reason to suspect there would be differences in the aerosol production or particles size but it is possible that MgSO_4 , could change the pattern of breathing, either through a response to its taste or tonicity or by changing the pattern of ventilation by reducing bronchospasm in asthmatics so caution is necessary in extrapolating these results into the clinical situation. The intended comparison in this study was the *in vivo* deposition in healthy adults to the predicted deposition using an adult pattern of breathing in an *in vitro* model[10]. There is no reason to believe that had it been possible to use small children in this study that there would have been a disagreement with the *in vitro* model using a suitable pattern of breathing for the age and size range[10]. Although *in vitro* data using breath enhanced nebulizers suggested that on a mg per kilogram of body weight small children would receive more medication than larger ones[29], *in vivo* data[30] where children over a wide size range inhaled the same dose of tobramycin showed virtually the same blood levels which would strongly imply a drug deposition normalized for size. The likely explanation for this discrepancy is that the definition of RF with a cut off of $\leq 5 \mu\text{m}$ is not applicable to children with a smaller upper airway. While *in vivo* data is sparse, modeling of the upper airway in infants[31] would imply that the definition of the RF should be particles much smaller, perhaps $\leq 3 \mu\text{m}$ [32] or even smaller[33]. This would be supported by the *in vivo* nuclear medicine deposition studies by Chua et al[19] who compared deposition from the traditional unvented

nebulizer over a large size range in infants and children with cystic fibrosis. Similar conclusions were reached in a deposition study using metered dose inhalers with valved holding chambers in infants[34]. The *in vitro* data for this for the delivery system used in this study[10] did employ varying definitions of RF and patterns of breathing based on size of the child and did suggest that the expected pulmonary dose for a breathing pattern of small child would be significantly less compared to an older child or adult.

In conclusion, the AeroNeb Go[®] vibrating mesh nebulizer coupled with the novel holding chamber, the Idehaler Pocket[®] did confirm the deposition predicted from an *in vitro* model using a pattern of breathing appropriate for healthy adult males was within expected limits but caution is necessary if extrapolating these results to small children with asthma. This device appears highly suitable and superior to conventional nebulizers with respect to the use in a clinical trial of inhaled magnesium in children with refractory asthma. The delivery system would be expected to deposit relatively high concentrations of MgSO₄ directly on the respiratory epithelium which, given the anatomical proximity, would be expected to have much higher concentrations of the drug around the smooth muscles of the airway than could have been safely achieved using systemic intravenous delivery. It is hoped that this system will facilitate efficacy of inhaled MgSO₄ in the treatment of refractory asthma in children while avoiding the frequent side effects seen with much larger systemic doses delivered intravenously.

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The Idehaler Pocket[®] was supplied at no cost by La Diffusion Technique, St Etienne, France

Author Disclosure Statement

No conflict of interest exists for any of the authors.

Legend for Figures

Figure 1 shows the AeroNeb Go[®] vibrating mesh nebulizer coupled with the novel holding chamber the Idehaler Pocket[®] in the configuration used in the *in vitro* study[10]. (Courtesy of La Diffusion Technique Francaise, St Etienne, France),

Figure 2 is an example of the first nuclear medicine image after nebulization illustrating the distinction between the regions of interest.

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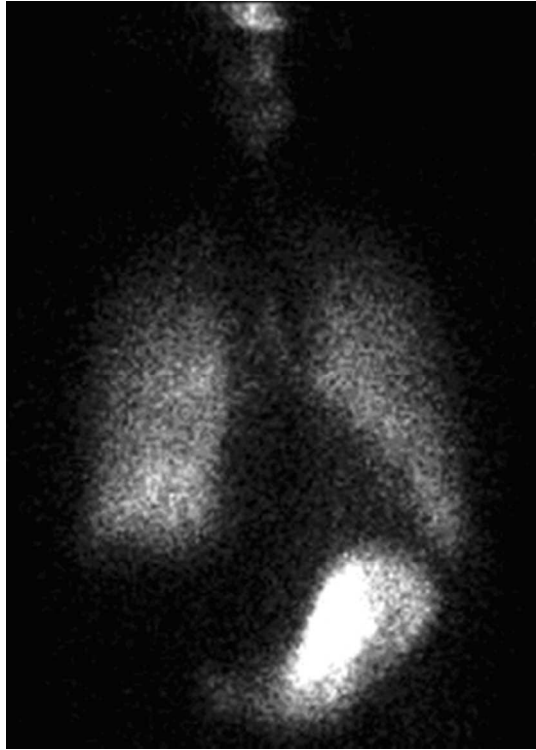


Figure 2 is an example of the first nuclear medicine image after nebulization illustrating the distinction between the regions of interest.
70x98mm (96 x 96 DPI)