

Pulmonary Delivery of Human IgG Antibody Using a Novel Digital Inhaler in a Rodent Animal Model

Jamie Balarashti¹, Cynthia Besch-Williford², Heidi Nelson-Keherly³ and Steven Kesten^{4*}

¹Aerosol Research and Engineering Laboratories Inc., Olathe KS, USA

²IDEXX BioResearch, Columbia, MO, USA

³CSSi LifeSciences, Glen Burnie, MD, USA

⁴Pneuma Respiratory, Boone, NC, USA

*Corresponding Author: Steven Kesten, Pneuma Respiratory, Boone, NC, USA.

Received: November 15, 2018; Published: December 06, 2018

Abstract

Background: Systemic delivery of monoclonal antibodies is used for lung cancer. Inhaled delivery may improve benefit-risk by delivering high local concentrations relative to systemic delivery. A study was designed to evaluate the ability of a novel digital breath-activated inhaled delivery system to deliver human IgG to the lower airways and alveoli.

Methods: Male Sprague Dawley rats were exposed to IgG aerosols via the digital inhaler in a 6-liter chamber. Six dose groups were exposed: water control, range-finding, low, high, high+blood sampling, and intraperitoneal dose groups. Active groups inhaled IgG for 45 or 135 minutes (IgG concentrations of 0, 5, 10 and 25 mg/mL). Animals were sacrificed no more than 4 hours post-exposure. Lung was scored for distribution and amount of bound antibody using a 0 - 4 immunohistochemistry grade: (0 = no significant labeling, 1 = minimal scattered to diffuse labeling, 2 = mild scattered to diffuse labeling, 3 = moderate diffuse labeling, 4 = marked diffuse labeling.

Results: Distribution and level of IgG labeling in the low dose was mild to moderate. High dose group labeling was considerably more pronounced with moderate IgG distribution in proximal and distal lung. Average immunohistochemistry score per tissue examined: control = 0.0, range-finding group = 1.3, low dose = 1.4, high dose = 2.4. Total score (all tissues examined): control = 0, range finding = 36.0, low dose = 38.5, high dose = 68.0.

Conclusions: IgG was distributed in proximal and distal lung tissues for the high dose animals and more moderate levels present for lower dose groups. The study demonstrated that a large molecule biologic could be delivered to the lower airways and alveoli of rats using the digital inhaler.

Keywords: Lung Cancer; Aerosol Drug Therapy; Inhalation Devices; Immunotherapy

Introduction

Several new drug therapies using monoclonal antibodies, such as nivolumab and pembrolizumab, have recently been developed for treatment against cancer [1]. These new drug therapies are IgG4 isotype antibodies that block a protective mechanism of cancer cells and allow the immune system to destroy those cancer cells [1]. Currently, antibody therapeutics are limited to needle-based injection deliver routes regardless of their use for systematic or local lung action. Pulmonary delivery to the target lung regions (lower airways and alveoli) has the potential benefit of increasing efficacy and reducing overall systematic dose, thus reducing associated side effects of the treatment.

Inhaled delivery of medications remains the mainstay for pharmacotherapy for obstructive lung disease [2,3]. A multitude of systems are available to delivery airway medications such as bronchodilators and inhaled corticosteroids. The delivery systems include pressur-

ized metered dose inhalers, soft mist inhalers, dry powder inhalers, ultrasonic nebulizers and jet nebulizers. Each has inherent advantages and disadvantages that relate to reliability of precise drug amounts to the lower airways and consistency of user performance [4,5]. For biologic agents, additional factors need to be considered; proteins delivered under pressure or heated to aerosolize will likely not remain intact and thereby have diminished effectiveness.

This study investigates the feasibility of using a novel fully digital breath-activated multidose inhaler using an electronically driven piezoelectric system that does not require pressure or heating for delivery. This breath-activated digital inhaler (BDI) has a reusable body with removable and disposable drug cartridges. The disposable cartridges are designed to provide single doses and multiple doses (i.e. 100 to 200 actuations) of aerosolized medication to the user and is suitable for a wide range of therapeutic inhalational drugs. The BDI can produce consistent and precise dosing. A study was therefore designed to assess the delivery of human IgG monoclonal antibodies, a surrogate for true monoclonal antibody therapies for disease, directly to the lower airways and alveolar regions of the murine lung.

Materials and Methods

An abbreviated description of the methods is described below. A full and detailed description is available as supplementary information.

Study groups

Male Sprague Dawley rats were divided into 6 dose groups (3 rats in each group, except for the intraperitoneal group (IP) which consisted of 2 animals): fresh-air control, range-finding group, low dose (3 mg/kg), high dose (15 gm/kg), high dose with 72-hour post-exposure blood plasma draws (high+blood), and intraperitoneal dose (150 ug/kg) (Table 1). Rats were approximately 17 weeks old and 275 grams when the first two dose groups were conducted.

Group	Animal model	Formulation	Exposure Duration (min)	Animals (#)	Blood draws
Control	Sprague-Dawley (m, 18 weeks)	Filtered DI Water	45	3	-
Range-finding	Sprague-Dawley (m, 18 weeks)	10 mg/ml hIgG, DI Water	45	3	-
Low	Sprague-Dawley (m, 18 weeks)	5 mg/ml hIgG, 0.001% Tween 80, 0.02% Antifoam A, filtered DI Water	135	3	-
High	Sprague-Dawley (m, 18 weeks)	25 mg/ml hIgG, 0.001% Tween 80, 0.02% Antifoam A, filtered DI Water	135	3	-
High + Blood Draws	Sprague-Dawley (m, 18 weeks)	25 mg/ml hIgG, 0.001% Tween 80, 0.02% Antifoam A, filtered DI Water	135	3	0, 1, 24, 72 hours
Intra-peritoneal	Sprague-Dawley (m, 18 weeks)	200 ug/ml hIgG, filtered DI water 250ul (50 ug Dose)	N/A	2	0, 2, 4 hours

Table 1: Human IgG challenge study test matrix.

Aerosol Generator

The aerosol generator used for the study was the PneumaHaler™ Breath-Activated Digital Inhaler (Pneuma Respiratory, Boone, North Carolina), which is being developed to deliver small and large molecules to the lung (Figure 1). The Breath-Activated Digital Inhaler (BDI) comprises a drug delivery cartridge with a vibrating plate piezoelectric sprayer embedded on the bottom of the cartridge, and a hand-held unit contacting a differential pressure sensor, batteries and a microprocessor that controls dose delivery, user notification, and dose counting. When the cartridge is mated to the hand-held body, electrical contact is made between the body containing the batteries and the piezoelectric sprayer embedded in the drug cartridge.



Figure 1: Breath-Activated Digital Inhaler (BDI) utilized in the study.

The (BDI) has side ports which draw ambient air during the inhalation cycle of the patient; the side ports were modified by attaching poly tubing to the side ports of the inhaler. The tubing allowed for metered control of all air flow through the inhaler. The BDI internal programming was modified to allow the device to fire in near continuous mode with approximately 4.2 seconds of actuation every 5.2 seconds (11.53 actuation/minute; 80.7% duty cycle). This modification was performed to the electronics control circuitry purely to increase inhalation chamber concentrations for the exposure and did not affect the output rate (ug/sec) or the output particle size distribution from the device. Initial characterization showed that this modification did not affect the BDI output particle size distribution. Total number of actuations for each of the 45 and 135 min exposures were approximately 520 and 1,560 respectively.

Aerosol Measurement

A TSI Aerodynamic Particle Sizer (APS) model 3321 (TSI Inc. Shoreview, MN) was used to measure all challenge aerosols in the exposure chamber during pre-characterization, characterization and animal exposure trials. A California Measurement’s PC-2AS real-time piezo mass impactor (California Measurements Inc. Sierra Madre, CA) was used to sample resident aerosols periodically in the exposure chamber during all trials.

Exposure System

The exposure system used was a cylindrical lexan whole-body small animal exposure system modified with inhaler induction port, isokinetic sample ports for monitoring the particle size real-time and triplicate exhaust path to ensure homogeneity in the cylindrical exposure chamber (Figure 2).

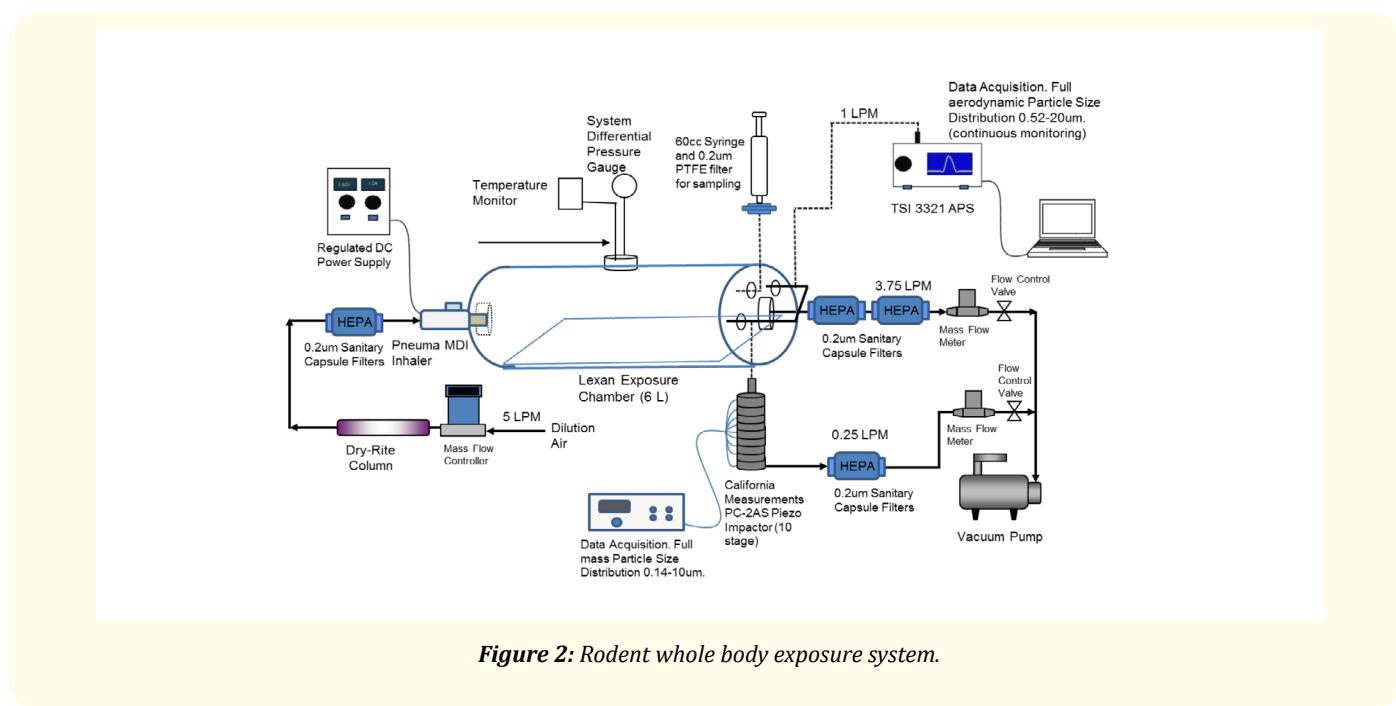


Figure 2: Rodent whole body exposure system.

System pre-characterization

Prior to animal studies, several chamber characterization exposures were conducted to determine the aerosol characteristics of exposure system and characterize the BDI delivery device. The several pre-characterization aerosol exposures used albuterol sulfate at 10 mg/mL for durations up to 45 minutes. These data were used to calculate the combined delivery efficiencies of the system and to estimate overall dosing for the hIgG trials.

Product formulation

Albuterol sulfate solution (10 mg/mL total dissolved solids) was used for characterization of the exposure chamber and inhaler delivery characterization pre-trials. Human immunoglobulin (hIgG) solutions used a carrier of 0.001% Tween 80 and 0.02% Antifoam A in filtered deionized water.

Aerosol delivery efficiency

Delivery efficiencies were calculated for the BDI using the pre-characterization results, and these initial delivery efficiencies were used to estimate exposure duration to reach target animal dose for the IgG challenges. The delivery efficiency was defined as the total suspended aerosol concentration divided by the BDI use rate. The BDI delivery efficiency values were averaged and used to estimate the exposure duration for the range-finding exposure group for the human IgG exposures.

Determination of rodent lower airway and alveolar dose

To maximize murine lower airway and alveolar deposition, a series of characterization trials were conducted with various hIgG formulations in the BDI. These data were used, in conjunction with rodent particle deposition models, to select the target dose concentration based on maximizing alveolar dose while minimizing/balancing the upper respiratory dose to the animal model. For the rodent, particle mass below 2.2 μm was considered as lower airway and alveolar deposition.

Particle characterization trials using the TSI APS were conducted with 5, 25, 50 and 75 mg/mL hIgG solutions to determine the relative deposition fractions, based on the particle size distribution data in the exposure chamber.

The estimated alveolar dose was determined by multiplying the average hIgG aerosol mass concentration by the APS measured alveolar fraction by the duration of the exposure and estimated minute-volume of the animal [7]. This process was repeated to calculate for the estimated upper respiratory dose for the rodent. The data was tabulated and compared to select a hIgG concentration that yielded a high alveolar dose (reduced exposure time) yet maintained an acceptable upper respiratory dose.

Histopathology

Rats were euthanized with isoflurane/ CO_2 . Five-micron lung and trachea sections were stained with hematoxylin and eosin. Additional sections were subjected to anti-human IgG immunohistochemistry with DAB-Peroxidase Brown (3,3'-Diaminobenzidine) chromogen to indicate deposition location of human IgG. Tissues were scored to distribution and amount of bound antibody (referred to as IgG label), using a modified standard grading system whereby 0 = no significant IgG labeling, 1 = minimal scattered to diffuse IgG labeling, 2 = mild scattered to diffuse IgG labeling, 3 = moderate diffuse IgG labeling and 4 = marked diffuse IgG labeling. Regions of the lung examined included trachea, bronchus, proximal bronchioles on the left or right lobes, proximal alveoli on the left or right lobes, distal bronchioles on the left or right lobes and distal alveoli (adjacent to the pleura) on the left or right lobes. A total score was calculated from the left and right lobe scores.

Saphenous vein sampling

Plasma from rats in the 2nd high dose group and the intraperitoneal (IP) dose group were collected and tested in two replicates at a 1:2 dilution by a micro-bead based IgG-capture assay using the Milliplex MAP kit (EMD Millipore, HGAMMAG-301K).

For the IP group hIgG concentrations, blood was collected at time 0. After IP hIgG intraperitoneal injection, blood was collected 2 and 4 hours intervals. For the high dose inhaled group, sampling occurring at time 0, 1, 24 and 72 hours.

Results

System Pre-Characterization

The average particle size distribution (PSD) by mass concentration for the entire 45-minute trial show a relatively monodispersed aerosol with a Mass Median Aerodynamic Particle Diameter (MMAD) of 2.37 μm with a Geometric Standard Deviation (GSD) of 1.75 μm (Figure 3).

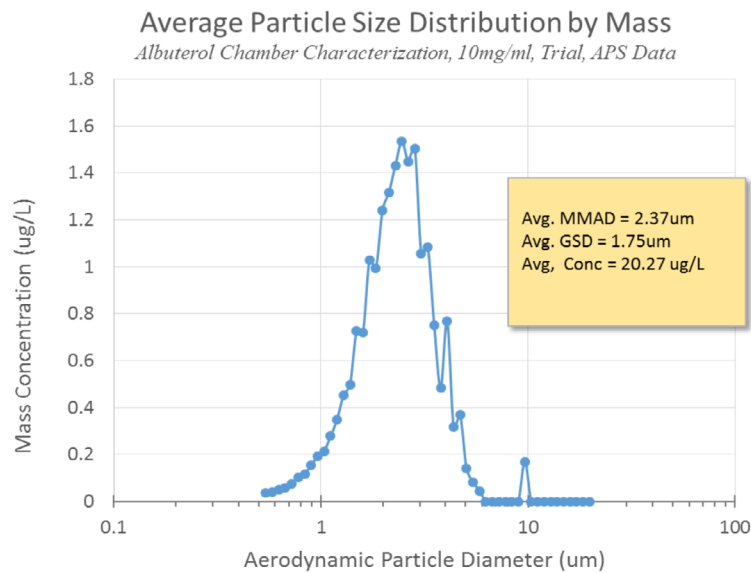


Figure 3: Particle size mass distribution for albuterol delivered via the BDI (10mg albuterol/mL).

Cumulative aerosol mass concentration (ug/L) and MMAD values (um) are plotted for the entire 45 minutes trial to show exposure chamber and aerosol generator consistency and stability. Figure 4A shows the cumulative concentration with a 5-period moving average overlay, while figure 4B shows the same data for the MMAD over the entire 45-minute characterization challenge.

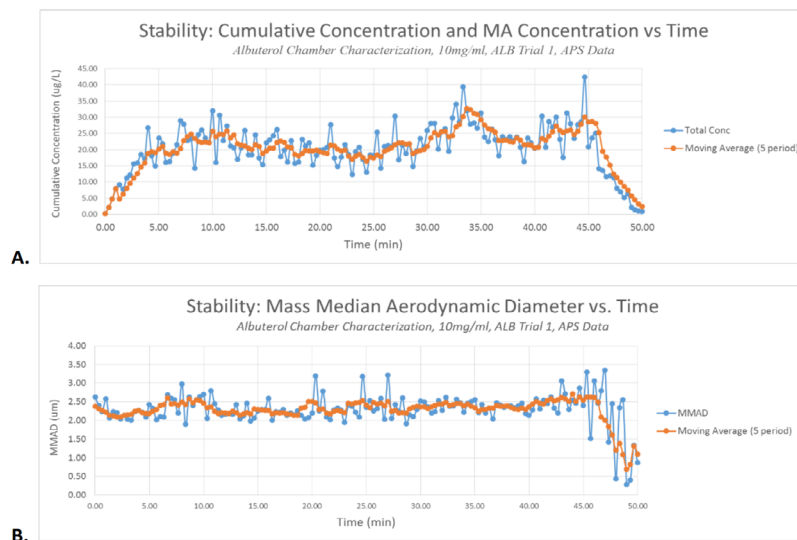


Figure 4: Mass concentration (A) and MMAD stability (B) of albuterol in the inhalation chamber during BDI delivery (albuterol 10 mg/mL).

Aerosol delivery efficiency

The test data showed that concentrations within the chamber were stable over time. The calculated delivery efficiencies for the BDI with albuterol (10 mg/mL) was high and showed a delivery efficiency of 21.4% and 20.9% for each trial respectfully.

Determination of rodent lower airway and alveolar dose

Based on the PSD data and deposition model, maximum alveolar deposition occurred with 5 mg/mL hIgG solution with an estimated 34.2% of the total inhaled dose depositing into the alveolar region of the pulmonary system. The predicted alveolar mass fraction decreased with increasing hIgG concentration and showed an estimated alveolar deposition fraction of 15.9% at a hIgG concentration of 75 mg/mL. The predicted alveolar dose for 75 mg/mL hIgG solution decreased, when compared with 50 mg/ml hIgG solution, due to the accompanied particle size increase, thereby showing the upper limit of simply increasing the inhaler hIgG solution concentration to yield increased alveolar delivery.

Based on these results, an upper hIgG solution concentration of 25 mg/mL was used for the animal study (high dose group). This dose level yielded a substantially higher alveolar dose compared with the 5 mg/mL concentrations, without significantly increasing upper respiratory deposition.

Exposures

Rats were exposed to aerosolized human IgG using the BDI with resultant chamber concentrations of approximately 54 ug/L air (range finding, 10 mg/mL, 45 minutes), 32 ug/L air (low dose, 5 mg/mL, 135 minutes) and 135 - 145 ug/L air (high dose, 25 mg/mL, 135 minutes) for a single exposure in the inhalation chamber.

Post-exposure dose calculations

Initial low dose group dose targets were 1,000 ug hIgG total inhaled dose. Calculations showed that the exposures were overestimated, and that actual challenge dosing was 1,344 ± 37 ug total inhaled hIgG dose. High dose target was 5,000 ug total inhaled dose and post-exposure results showed that the actual total inhaled dose was 5,596.1 ± 143 ug hIgG. The second high dose group dosing was 5,652.8 ± 537 ug hIgG.

Histopathology

A summary of histological and hIgG immunohistochemistry evaluation scores for the control, dose-range, low-dose and high-dose groups are displayed in table 2. The control group showed no hIgG labeling in proximal bronchioles and alveoli. Labeling in the dose-finding group was minimal to mild and scattered to diffuse in proximal bronchioles and alveolar sacs. Labeling intensity and distribution were decreased in distal bronchioles and alveoli. The distribution and level of IgG labeling in the low dose group was mild to moderate in proximal and distal lung tissues. Distribution of IgG to the distal alveoli was more consistent than in the dose-range group. Tracheal labeling in this group was also less than that observed in the parenchyma. A feature in this group not observed in the control and dose-finding lungs was an increase in the presence of lymphocytes egressing from blood vessels into the interstitial spaces around blood vessels.

	Control		Range Finding		Low Dose		High Dose	
	# Changes	Average Score	# Changes	Average Score	# Changes	Average Score	# Changes	Average Score
Lungs								
H&E								
Lymphoid aggregates, peri-bronchiolar	2	2.0	2	2.0	3	2.0	3	2.0
Atelectasis	2	1.5	2	1.5	1	2.0	1	2.5
Infiltrates, mast cell/lymphocytes	0		2	1.5	1	1.0	2	2.0
Hemorrhage	1	1.0	1	1.0	1	1.0	1	1.0
IgG Label								
Trachea	0		3	0.8	3	0.8	2	1.5
Bronchus	0		1	2.0	0		1	1.0
Bronchioles (left) - proximal	0		3	1.7	3	1.5	3	3.0
Bronchioles (left) - distal	0		3	0.8	3	1.2	3	2.3
Bronchioles (right) - proximal	0		3	2.0	3	2.0	3	2.8
Bronchioles (right) - distal	0		3	1.0	3	1.3	3	2.3
Alveoli (left) - proximal	0		3	1.3	3	1.7	3	3.0
Alveoli (left) - distal	0		3	0.8	3	1.3	3	2.3
Alveoli (right) - proximal	0		3	2.0	3	2.0	3	2.8
Alveoli (right) - distal	0		3	0.8	3	1.0	3	2.7
Total Tissues Per Group	30		28		27		28	
Total IHC Tissues Labeled	0		28		27		28	
Total IHC Score	0.0		36.0		38.5		68.0	
Average Score/Sample	0.0		1.3		1.4		2.4	

Table 2: Summary of histologic and hIgG immunohistochemistry evaluation.

In the high dose group, IgG labeling in lung parenchyma was mild to moderate with better distribution to distal portions of the lung than the labeling observed in the range finding and low group. Figure 5 illustrates IgG staining in the lung of a high dose treated rat. Like changes observed in the low-dose group, lymphocytes infiltrated multiple perivascular interstitial spaces. This inflammatory response was milder and occurred in fewer rats.

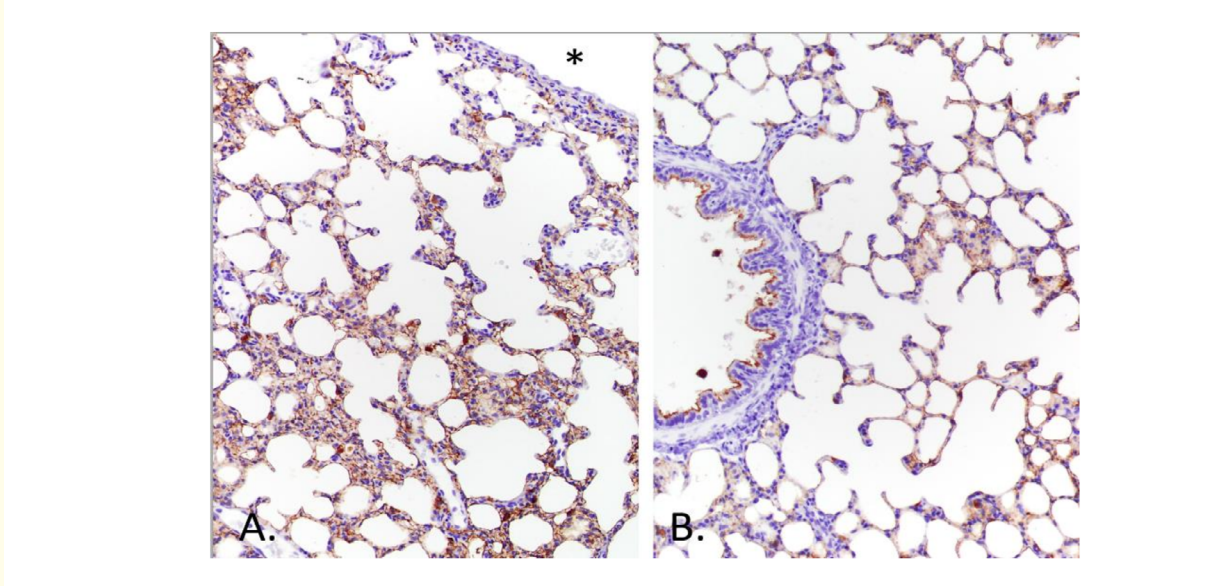


Figure 5: Immunohistochemical staining of hIgG in high dose group; distal alveoli and bronchiole labeled images, 20x magnification.

Plasma hIgG concentrations

In the rats dosed by inhalation, levels of total hIgG increased over time. The levels detected at 24 hours post-exposure approximated in the high dose inhalation group (5,500 ug dose) those detected in the plasma of rats dosed with hIgG by intraperitoneal injection (50 ug dose) at both 2 and 4 hours (Figure 6).

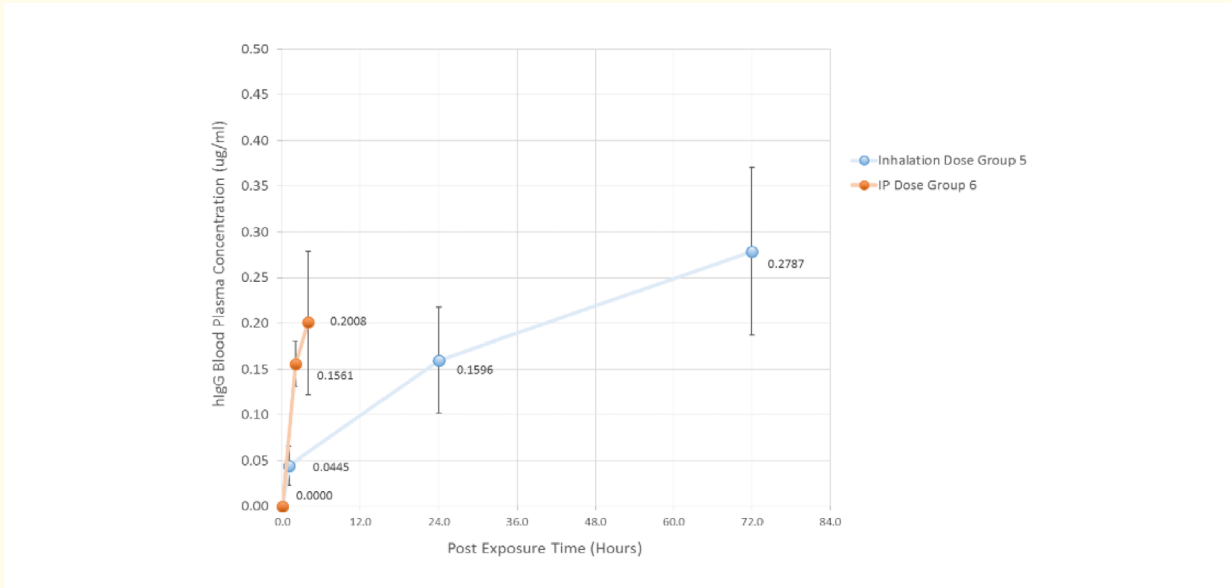


Figure 6: Plasma hIgG concentrations (mean ± SD) in the high dose inhalation (5,500 ug dose) and intraperitoneal (50 ug dose) groups.

Discussion

Biologic therapeutics based on monoclonal antibodies have conclusively demonstrated to be effective and have received regulatory approval. In particular, anti-TNF inhibitors for several systemic diseases and anti-PD1 antibodies for cancer have obtained global regulatory approval and are medically accepted alternatives for serious and life-threatening diseases [1,8,9]. These biologic interventions require systemic delivery and repeated dosing, which at least for anti-TNF inhibitors may be over the lifetime of the patient. Additionally, the incidence of adverse effects is common with several being serious and potentially life-threatening [10-13]. Opportunities to improve the benefit to risk profile should continuously be explored but may be product and indication specific. An example is the possibility of local delivery (i.e. inhaled) of anti-PD1 antibody immunotherapy therapy for non-small cell lung cancer.

The recent development of a novel fully digital inhaled delivery system (PneumaHaler™ Breath-Activated Digital Inhaler (BDI)) provided the opportunity to evaluate the feasibility of inhaled delivery of an antibody-based treatment to the lower airways. Unlike propellant driven multi-dose inhalers, the droplets from the BDI are generated having little to no intrinsic velocity from the aerosol formation process and are inspired into the lungs solely by the user's incoming breath passing through the mouth tube. The design features allow for precise dosing (prerequisite for biologic treatments), dose adjustments (due to the digital software-based system) and multiple dosing (if needed). Given the absence of a pressurized system and heating to generate the aerosol, proteins are expected to be delivered intact (i.e. not denatured).

Prior to the rodent intervention aspect of the current study, initial feasibility of the inhalation chamber was demonstrated. The BDI was capable of emitting consistent dosing over a prolonged period as shown by steady state albuterol concentrations within a reasonable range. The second phase was to expose rats to aerosolized human IgG (hIgG) using the BDI with exposure times of 45 minutes or 135 minutes and hIgG concentrations in the formulations of 0, 5, 10 and 25 mg/mL. Animals were sacrificed for histologic evaluation of the lungs no more than 4 hours after a single aerosol exposure of 45 or 135 minutes exposure in an inhalation chamber.

Immunohistochemistry on rat lung and trachea showed moderate IgG distribution in proximal and distal lung tissues for the high dose animals and less IgG deposits in the lower dose groups. Minor inflammatory cell infiltrates were noted and there did not appear to be any other short-term adverse effects on the animals.

Immunotherapy for non-small cell lung cancer is now an accepted option under specific circumstances within treatment guidelines [1]; however, adverse events are clinically important and must be considered in prescribing decisions. For example, the adverse events for pembrolizumab include pneumonitis, hepatitis, colitis, endocrine gland dysfunction (thyroid, pituitary, adrenal, pancreas), nephritis and kidney failure, myocarditis, myalgia, arthralgia, encephalitis and anemia [10]. Similar adverse events are attributable to compounds with the same mechanism of action such as nivolumab [11]. One possible approach to create a more favorable benefit-risk profile would be to obtain a higher ratio of local/systemic exposure; however, such a hypothesis is based on several assumptions that have yet to be proved. Nevertheless, an initial step is to assess whether such biologic agents can be delivered to the lower airways, which has now been demonstrated through this study. Sapilidis, *et al.* have also considered the feasibility of inhaled delivery and reported the results of their evaluation of the design factors within a nebulized system that would permit aerosolization of three immunotherapeutic agents [14]. Maillet, *et al.* assessed the aerodynamic, pharmacologic and immunologic properties of a chimeric IgG1 targeting epidermal growth factor receptor following nebulization and determined that it was feasible although IgG aggregation was described with jet and ultrasonic nebulizer systems [15]. However, this was not observed with the mesh nebulizer, which is a similar technology to that used with the BDI.

The application of biologic therapies can be limited by factors including but not limited to the following: need for systemic delivery to obtain local (target organ) effects, bioavailability (i.e. first-pass hepatic metabolism), achieving optimal concentrations relative to expected adverse effects, the overall safety profile, and feasibility/tolerability of multiple doses over extended periods of time. Direct organ delivery offers an option that addresses several of the aforementioned issues. Direct delivery may be achieved by intravascular targeting

to the affected organ or in the case of the lungs, by inhalation. In the current study, delivery was achieved by inhalation. In the BDI high-dose group of rats, systemic levels detected at 24 hours post-exposure approximated those detected in the plasma of rats dosed with hIgG by intraperitoneal injection at both 2 and 4 hours; however, it is relevant to note that the lung dose (5,500 ug) was over 100 times that of the intraperitoneal dose (50 ug). Other investigators have described the low systemic bioavailability (approximately 1 to 4%) of monoclonal antibodies delivered by inhalation in a non-human primate model [16]. Of note, plasma levels in the high dose group were detected at 72 hours that were above 24-hour levels suggesting lung retention of hIgG, which may have favorable clinical implications.

The BDI represents an advance in inhaler development through full integration of digital technology to create the capability of delivering different therapies, including large molecule biologic therapeutics. The system evaluated does not require pressurization or heating to generate and expel an aerosol. Ejection occurs with minimal intrinsic velocity such that delivery follows entrained air. The software control can be adjusted to alter volumes and timing without redesigning the entire system. Such features and the findings from this study provide encouragement regarding continued investigations of inhaled immunotherapy for highly morbid lung diseases such as cancer.

Limitation of the Study

Study limitations must be acknowledged. While hIgG was detected in the lower airways and alveoli, an assessment of biologic activity was not performed. The actual disease effective dose relative to systemic delivery was not studied; therefore, clinical conclusions are only speculative. While only a minor inflammatory reaction was observed in delivering human IgG to the rat, additional studies will be required to observe whether such an observation was purely related to interspecies aspects rather than the inhaled delivery itself. Finally, the lung dose is calculated from the concentrations in the chamber and assumed rat minute volumes but still is an estimated lung dose.

Conclusion

The presented study demonstrated the feasibility of the testing inhalation chamber using the BDI device in that a prolonged and repeated ejection of a test drug substance could achieve stable steady state concentrations. The second phase of the study proved that a large molecule biologic compound could be delivered to the lower airways of rats using the BDI device. The implications are that, like currently available medications for obstructive lung disease, direct target delivery to the lungs is feasible through this digital inhaled platform for biologic compounds that may improve the overall benefit to risk profile for specific lung diseases such as non-small cell lung cancer and potentially for immune-related diseases of the lung. Secondary clinical implications may be improved cost-effectiveness through the avoidance of direct systemic administration (intravenous, subcutaneous) and perhaps through lower dosing. In conclusion, these studies indicate that the BDI may be a solution for pulmonary delivery of monoclonal antibody therapies for targeted delivery and treatment of oncologic or immune mediated lung diseases. Future studies will need to be performed to evaluate the effectiveness on actual disease models.

Author Contributions

J. Balarashti: Study design, data acquisition, analysis, interpretation, drafting the work.

C. Besch-Williford: Study design, data acquisition, analysis, interpretation, drafting the work.

H. Nelson-Keherly: Study design, analysis, interpretation, drafting the work.

S. Kesten: Analysis, interpretation, drafting the work.

Funding

The study was sponsored by Pneuma Respiratory, Inc.

Author Disclosure Statements

J. Balarashti is an employee of Aerosol Research and Engineering Laboratories Inc. (ARE). ARE performs paid services for Pneuma Respiratory. C. Besch-Williford is an employee of IDEXX BioResearch. IDEXX BioResearch performs paid services for Pneuma Respiratory. H. Nelson-Keherly is an employee of CSSi LifeSciences. CSSi LifeSciences performs paid services for Pneuma Respiratory. S. Kesten is an employee of Pneuma Respiratory.

Bibliography

1. Bironzo P and Di Maio M. "A review of guidelines for lung cancer". *Journal of Thoracic Disease* 10.13 (2018): S1556-S1563.
2. Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for the Diagnosis Management, and Prevention of Chronic Obstructive Lung Disease 2019 Report [internet] (2018).
3. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention [internet] (2018).
4. Price D., *et al.* "Inhaler competence in asthma: common errors, barriers to use and recommended solutions". *Respiratory Medicine* 107.1 (2013): 37-46.
5. Lavorini F., *et al.* "Effect of incorrect use of dry powder inhalers on management of patients with asthma and COPD". *Respiratory Medicine* 102.4 (2008): 2593-2604.
6. Menache MG., *et al.* "An empirical dosimetry model of aerodynamic particle deposition in the rat respiratory tract". *Inhalation Toxicology* 8.6 (1995): 539-578.
7. Guyton AC. "Measurement of the respiratory volumes of laboratory animals". *American Journal of Physiology* 150.1 (1947): 70-77.
8. Singh JA., *et al.* "Guideline for the treatment of rheumatoid arthritis". *Arthritis and Rheumatology* 68.1 (2016): 1-26.
9. Pugliese D., *et al.* "Anti-TNF-alpha therapy for ulcerative colitis: current status and prospects for the future". *Expert Review of Clinical Immunology* 13.3 (2017): 223-233.
10. "Keytruda (pembrolizumab) Package Insert" (2018).
11. "Opdiva (nivolumab) Package Insert" (2018).
12. "Humira (adalimumab) Package Insert" (2018).
13. "Enbrel (etanercept) Package Insert" (2018).
14. Sapalidis K., *et al.* "Inhaled immunotherapy administration for lung cancer efficient? Certainly possible". *Journal of Cancer* 9.6 (2018): 1121-1126.
15. Maillet A., *et al.* "Aerodynamical, immunological and pharmacological properties of the anticancer antibody cetuximab following nebulization". *Pharmaceutical Research* 25.6 (2008): 1318-1326.
16. Sakagami M., *et al.* "Quantifying Monoclonal Antibody Disposition Following Aerosol Administration: Systemic and Airway Exposure in Non-human Primates". *Respiratory Drug Delivery Europe* (2015).

Volume 8 Issue 1 January 2019

©All rights reserved by Steven Kesten., *et al.*