A Miniaturized Inhalation Tower to Deliver Small Amounts of API to Conscious Rats.

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Abstract

Historically, delivering aerosol dry powders to the lungs of pre-clinical species has been challenging. Typically the available techniques require using large amounts of API and/or invasive techniques such as intratracheal administration of the drug. In addition this technique generally requires anesthesia and its incumbent physiological and pharmacological concerns. Besides these less than desirable parameters, the current techniques are not entirely representative of human clinical inhalation and delivery of compound to the lungs. To advance Merck's current capabilities for delivering both liquid and dry powder aerosols to conscious rats, we have developed a state-of-the-art miniaturized method for aerosol delivery that requires only small amounts of API which are delivered to conscious rats. These advances were achieved by integrating the engineering expertise from Buxco Electronics, pulmonary expertise from Merck's Inhalation Platform, and the overall expertise for inhaled drug delivery from Merck's Respiratory Product Development Department. The system that was developed and validated is known as the Buxco Inhalation Exposure Tower or BIET. The BIET consists of a nose-only exposure system attached to an aerosol chamber to minimize skin and fur contamination, eliminates the need for anesthesia, and minimizes compound use. BIET can also be used to measure in real-time changes in respiratory physiology during drug delivery. Measuring these respiratory physiological parameters provides an index that allows the identification of potential liabilities that may be associated with the inhaled novel chemical entities while at the same time providing real time minute ventilation during drug delivery. The ability to measure real time ventilation is an advance over typical inhalation delivery systems. Now the delivered dose (DD) of compound no longer has to be calculated using estimated respiratory minute ventilation (RMV) based on the animals body weight but can be measured in real time. In the present studies, the Allay™ restraint collar[1] was used in conjunction with the nose-only plethysmograph to measure ventilation in rats exposed to a variety of air flows in the range traditionally used in larger safety and toxicology towers. This allowed us to assess the effect of different airflows through the BIET and compare the RMV in the chambered animals compared to animals under normal ventilation conditions measured as a control outside the unit but in plethysmographs. Male Wistar rats (143-213 g) were placed in the Allay™ restraint collar/nose-only plethysmograph for 30 min per experiment while breathing room air outside of the BIET. Tidal volume (Vt, ml), respiratory rate (f, breaths/min) and minute ventilation (RMV, ml/min) measured on a breath-by-breath basis and showed remarkable stability over this 30 min control period. RMV decreased slightly over time as the rat accommodated to the restraint. Average values for Vt (2.10 ± 0.14, ml), f (131 ± 8, breaths/min) and RMV (183 ± 34, ml/min) were within values reported in the literature. When the rats were placed in the BIET, on-line ventilation data was obtained as the rats breathed air in the tower at different flow rates that would be used during exposure of the rats to inhaled drugs. Values for RMV at air flow rates ranging from 2.5-8.0 liters per minute ((LPM) circulated through the tower were reduced when compared to RMV values obtained during normal ventilation outside of the tower (183 ± 34, ml/min in the BIET, 259 ± 41, ml/min outside). These differences were not statistically different. When traditional methods were used to calculate RMV based on animals weight for the animals on study, these calculated RMV values were greatly reduced in comparison to those actually measured. In conclusion, the BIET tower using the Allay™ restraint collar and nose-only plethysmograph offers an advantage over conventional systems by reducing drug requirements; avoid reliance on estimations of RMV for calculating DD and eliminating anesthesia. This miniaturized tower system yields high quality ventilation data for an extended period of time and can be used to assess the respiratory effects of drugs given by nose-only inhalation through the inhalation tower. The ability to maintain normal ventilation over a large range of airflow through the tower enables Merck to be able to use small amounts of API for evaluations using this tower in conscious rats.

1 House, A., R. Shafer, Y. Shemesh, J. Lomask, R. W Chapman, P. J Mauser Use of the ALLAY™ Restraint Collar to Facilitate the Measurement of Ventilation in Conscious Rats. Am. J. Respir. Crit. Care Med. A4049, 2013

Introduction

- Aerosol delivery of dry powders to the lungs of pre-clinical species has been historically challenging. Typically it requires using large amounts of API and invasive techniques such as intratracheal administration under anesthesia. These techniques are not entirely representative of clinical inhalation.
- In order to advance Merck's current capabilities for delivering both liquid and dry powder aerosols to conscious rats, we have developed a state-of-the-art miniaturized method of aerosol delivery using small amounts of API delivered to conscious rats by integrating the expertise from Buxco Electronics, Merck's Inhalation Platform, and Merck's Respiratory Product Development Department.
- The system developed and validated is the Buxco Inhalation Exposure Tower (BIET). The BIET is a nose-only exposure system that minimizes skin and fur contamination, avoids anesthesia, and minimizes compound use. In addition BIET has the ability to measure in real-time changes in respiratory physiology during delivery.

Methods

Animals:-

- Experiments were performed on male Wistar rats ranging in weight from 145-220 g.
- The rats were allowed to acclimate to the restraint for 5 days before measurements were obtained.

Allay™ Restraint Collars:-

➢ **Step 1:**

- The Allay™ restraint collars are color coded to fit different size rats (Fig 1). The appropriate size collar was selected to fit snugly over the rat's neck, just behind the ears and in front of the shoulders.

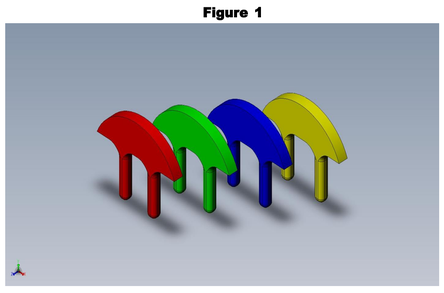
➢ **Step 2:**

- The rat was positioned in the plethysmograph chamber and the Allay™ restraint collar was inserted through the slot at the top of the plethysmograph and positioned over the neck of the rat just behind the ears (Fig 2).

➢ **Step 3:**

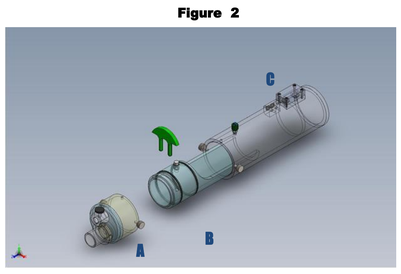
- The nose-cap was attached to the front end of the plethysmograph until the O-rings locked in place. Care was taken to ensure that the nose of the rat passed through the latex nose-seal (Fig 2). The rear of the plethysmograph was then sealed until the O-rings locked in place.

Figure 1



Allay™ restraint collars for use in rats. Yellow is the largest 0.75", blue is intermediate 0.70", green is smaller 0.65"and red is the smallest. 0.060"

Figure 2



Nose-only plethysmograph adapted for use with the Allay™ restraint: (A): nose-cap with latex nose seal, **(B)**:Allay™ restraint collar, **(C):** wire mesh screen

Experiment design:-

- Experiments were performed to evaluate the quality of the ventilation data while exposing the rats to various flow rates in the BIET tower.

➢ **Step 1:**

- Ventilation was measured for 30 min while breathing room air. Rats were then placed in the tower. Fig (3)

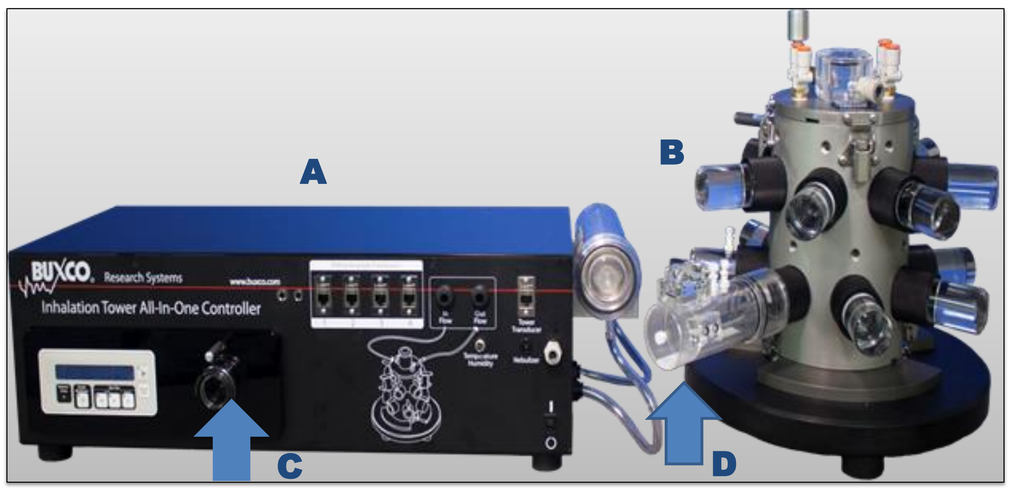
➢ **Step 2:**

- The flow rate was set for a pre-determined flow rate. Ventilation measured over a 20 min period while breathing air from the BIET at 7 flow rates ranging from 2.5 to 8.0 L/min.
- Ventilation in the tower was compared to ventilation breathing room air.

Measurement of Ventilation:-

- Airflow was measured as the pressure drop across a wire mesh screen placed in a hole in the wall of the plethysmograph using a differential pressure transducer (TRD5700) and volume signals were derived by integration of this airflow signal.
- Volume calibrations were performed using the FinePointe Calibrator.
- With the rat in the plethysmograph, tidal volume (Vt, ml), respiratory rate (f, breaths/min) and minute volume (RMV, ml/min) were measured for each breath and the average of this data was displayed every minute.
- All data was generated using Buxco's FinePointe™ System.

Figure 3



All-in One Controller and BIET:
(**A**): All-in-Once Controller Unit (**B**): Tower (**C**): FinePointe Calibrator (**D**) Plethysmograph

Results

Table 1

- **Table 1 shows ventilation at different flows rates while the pressure in the tower is maintained at zero.**
- **These flow rates are approximately 1.5 X and 5.0 X the average RMV of a 200 g rat.**

Flow (LPM)	LPM/port	Vt (ml)		f (breaths/min)		MV (ml/min)		BWT
		Air	Tower	Air	Tower	Air	Tower	(g)
2.5	0.5 *	2.18 ± 0.25	1.87 ± 0.24	126 ± 9	117 ± 10	265 ± 26	212 ± 26	213 ± 5
3.0	0.6	1.68 ± 0.22	1.41 ± 0.15	147 ± 6	107 ± 6	217 ± 77	136 ± 48	148 ± 2
4.0	0.8	2.09 ± 0.21	1.92 ± 0.21	135 ± 5	110 ± 5	249 ± 26	184 ± 25	147 ± 2
5.0	1.0	2.16 ± 0.06	1.62 ± 0.13	125 ± 7	92 ± 4	282 ± 35	149 ± 18	143 ± 3
6.0	1.2	2.06 ± 0.13	1.96 ± 0.22	138 ± 8	117 ± 10	286 ± 34	240 ± 43	145 ± 1
7.0	1.4	1.98 ± 0.07	1.88 ± 0.67	141 ± 12	98 ± 4	272 ± 18	118 ± 3	148 ± 2
8.0	1.6	2.71 ± 0.70	1.87 ± 0.24	104 ± 8	105 ± 3	239 ± 72	240 ± 72	155 ± 3

*** The flow coming from the port is equal to Flow/# of ports being occupied.**

Table 2

- **Table 2 is a comparison of calculated Respiratory Minute Volume (RMV) to measured RMV**
- **RMV = Respiratory Minute Volume (L/min.) = (0.499 (BW)^0.809)[1]**

	Air		Tower	
LPM/port	Calculated RMV	Measured RMV	Calculated RMV	Measured RMV
	(L/min)	(L/min)	(L/min)	(L/min)
0.5	0.129	0.265	0.129	0.212
0.6	0.090	0.217	0.090	0.136
0.8	0.089	0.282	0.089	0.184
1.0	0.087	0.249	0.087	0.149
1.2	0.088	0.286	0.088	0.240
1.4	0.090	0.272	0.090	0.118
1.6	0.094	0.281	0.094	0.204

[1]Bide, R.W., Armour, S.J., and Yee, E., Allometric respiration/body mass data for animals to be used for estimates of inhalation toxicity to young adult humans. J.Appl. Toxicol. 20 (4):273-290, 2000)

Conclusion

- **The calculated RMV for rats restrained and breathing room air or from the tower underestimates the actual measures RVM.**
- **Measured RMV is consistent over the range of flows evaluated.**
- **The flow range at the port (0.5 to1.6LPM) covers a range in flow that is recommended to clear exhaled atmosphere and avoid rebreathing and oxygen depletion.**

Discussion

- **The BIET tower using the Allay™ restraint collar and nose-only plethysmograph offers an advantage over conventional systems that rely heavily on estimations of RMV for calculating DD. This miniaturized tower system yields high quality ventilation data for an extended period of time and can be used to assess the respiratory effects of drugs given by nose-only inhalation through an inhalation tower. The ability to maintain normal ventilation over a large flow range in the tower will enable Merck to be able to evaluate small amounts of inhaled API in conscious rats.**