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RESEARCH ARTICLE

Development of an inhalation chamber and a dry powder inhaler device for administration of pulmonary medication in animal model

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Abstract:

Context: Pulmonary route of administration is becoming more popular for drug delivery in pulmonary tract and lungs for local and systemic actions.

Objective: A dry powder inhaler (DPI) for delivery of dry powder and a nose-only inhalation chamber for small animals that can be used with nebuliser/DPI were designed.

Materials and methods: The inhalation chamber was made with a polypropylene-rectangular box and centrifuge tubes. DPI was made of a polypropylene tube. Micronized voriconazole and voriconazole solution were used for DPI and nebulizer, respectively, for both *in vitro* and *in vivo* studies.

Results: *In vitro* drug deposition from nebulizer was found to be 11–26% w/w and that from DPI was 42 to 57% w/w depending on experimental set up. Uniform deposition across all the inhalation ports was observed irrespective of the methods. Respirable fraction (RF) varied from 34 to 73% in case of nebulizer and from 47 to 54% in case of DPI. *In vivo* deposition of voriconazole in lungs was found to be 80–130 µg/g of lung tissue in case of DPI and 40–68 µg/g of lung tissue in case of using nebulizer.

Discussion: DPI designed was efficient in fluidizing powder bed and dispensing dry powder for inhalation. The inhalation chamber designed was efficient in uniformly distributing drug in various inhalation ports of the chamber.

Conclusions: The DPI and inhalation chamber designed can be successfully used for inhalation study with multiple animals especially mice.

Keywords: Inhalation device, dry powder inhaler, pulmonary drug delivery, aerosols, *in vivo* lung deposition, animal model

Introduction

Pulmonary route of administration is increasingly getting more attention for delivery of therapeutic molecules to treat local infection in pulmonary tract and lungs as well as for systemic action[1,2]. This route will receive more importance in the coming years due to the advent of therapeutically important proteins and genes to treat different disorders since this route provides more product stability, ease of administration, patient compliance as compared to many other routes of administration for the molecules. Efforts are currently on to design devices with superior performance and a number of devices already launched in the market in recent times[3,4]. Sometimes, it is required to assess the efficiency of drug as well as formulated inhalation product in animal models prior to use in humans. However, requirement of a suitable apparatus for testing of inhalation products in animal models remains a challenge for most of the researchers.

Researchers have reported chambers for full body exposure of animals[5–9]. However, full body exposure causes absorption of the drug from the total body surface instead of desired nose-only administration, which might complicate the dosimetry[10]. Another nose-only inhalation chamber was reported by a research group

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from University of Alberta to deliver aerosolized aqueous compounds to the respiratory tract of mice[11]. However, based on the deposition study the authors commented that large quantities of drug might be required for achieving adequate drug levels using that setup. Some commercial products are available for intratracheal administration such as Penn-Century microspray aerolizer which requires the insertion of a thin tube in the intratracheal region[12,13]. However, this method is stressful and requires positive pressure and anaesthetizing animals. Inhalation under positive pressure is different from inhalation under normal breathing pattern and hence the findings are difficult to correlate with those from animals in normal state[14]. Another challenging aspect of animal study for pulmonary medication involving dry powder is that the devices used for humans cannot be used directly for animals. Researchers have reported a hand-held nose-only apparatus for delivery of microparticles to mice[10]. However, this device is suitable for use in single animal at one-go and also the holding of an animal at the delivery port might be difficult without anaesthetizing the animal. Another nose-only inhalation chamber was reported for testing the exposure of needle shaped respirable nylon fibres[15]. Few researchers have also used Cannon nose-only inhalation chamber[16,17].

Here we have reported a design of an apparatus which can be satisfactorily used for administration of both dry powder and nebulization to conscious animals without disturbing the normal breathing pattern. The simple design requires a plastic chamber to accommodate tapered plastic tubes which hold individual animals. The design is versatile and is easily adaptable for different animals and of different sizes. Further, we have reported a device that can be used as a dry powder inhaler (DPI) for administration of medication to animals in dry powder form. This DPI or a conventional nebulizer can be mounted to the inhalation chamber for administering suitable medication. Voriconazole has been used as a model drug for the study. Both the developed devices were evaluated by *in vitro* and *in vivo* studies. Blend of micronized voriconazole with lactose was used for evaluation of the inhalation chamber with DPI and equivalent dose of voriconazole as solution was used for evaluation of chamber through nebulization route.

Materials and methods

Materials

Voriconazole was generously gifted by Dr. Reddys Laboratories Ltd. (Hyderabad, India). Lactose monohydrate was purchased from LobaChemie (Mumbai, India). Both voriconazole and lactose monohydrate were micronized in an air jet mill (Midas micronizer, M-50, Microtech Engineering Corporation, Mumbai, India). Hydroxypropyl beta-cyclodextrin (HPβCD) was purchased from HiMedia Laboratories Pvt. Limited (Mumbai, India). A Salter NebuTech HDN Reusable Nebulizer with polyvinyl chloride connecting tubing (0.5 cm internal dia) was generously gifted by Saltar Labs (Arvin, USA). Polypropylene centrifuge tubes (50 ml) were purchased from Tarson India Pvt. Ltd. (Kolkata, India). Compressed air cylinder with a regulator (max. pressure ~2 kg-f/cm^2) and an adapter for attaching the nebulizer tubing were purchased from Refrigeration India (Kolkata, India). A polypropylene made rectangular box (23.5 × 13.5 × 3.5 cm) with a hinged top was purchased from Tarson India Pvt. Ltd. (Kolkata, India). All other chemicals used were of analytical grade.

Methods

Design of the inhalation chamber

The nose-only inhalation device for administration of nebulized aerosol to multiple mice in conscious state was designed by using cheap and easily available parts. The design was similar to as shown in Figure 1A. It consisted of a rectangular box (23.5 × 13.5 × 3.5 cm) made of polypropylene. A rectangular small chamber (dosing chamber) of a size of 3 × 13.5 × 3.5 cm was built inside the box by using two plates placed vertically at the middle of the box facing each other at 3 cm apart. The bottoms of the plates were fixed tightly with the box surface using adhesive. This made partitions at both sides of the dosing chamber. The tapered ends of centrifuge tubes (50 ml capacity) were cut to make holes so that only the noses of the animal (mice) can tightly pass through the holes. The tubes were arranged in the box by making holes in the outer face across the width of the box so that the tubes could pass through the holes. The tapered ends of the tubes were put in the central dosing chamber through holes made at the plates covering the dosing chamber. Three centrifuge tubes were placed at equal distance in each sides of the dosing chamber. The other ends of the centrifuge tubes were screw capped. A rectangular hole (3 × 1.5 cm) was made at the bottom of the outer face of the dosing chamber across the length of the box for the entry of the nebulizer/DPI delivery port. Another hole was made at the lid of the box just above the dosing chamber and opposite to the entry port, for exit of the gas flow from the nebulizer. Alternatively, this small hole remained closed during its use with a DPI.

Design of DPI delivery chamber

The schematic design of the DPI or the fluidization chamber is shown in Figure 1B. It was made of polypropylene made centrifuge tubes. The tapered end of the centrifuge tube was cut to make space for placing a long pipette inside the tube and a pipette teat was placed on the pipette for pumping air to fluidize the powder bed. The hole at the tapered end was sealed tightly to prevent air leak, however, alternatively, a circular plate was placed inside the tube as shown in the figure to define the total volume of fluidization of powder. The powder for delivery was placed inside the centrifuge tube and turned upside down so that the powder could rest on the cap of the centrifuge tube. A hole was made on the wall of tube for the delivery of powder in the inhalation chamber. This

Figure 1. (A) The schematic diagram of the fabricated dosing chamber. **A.** Centrifuge tubes, **B.** Dosing chamber, **C**. Entry port of nebulizer in the dosing chamber, D. Exit port of the aerosol, **E.** Box cover, **F.** Hook in the box body, **G.** Hook in the box cover for tight attachment with the box, **H.** Wide end of centrifuge tube for placing the animal inside the tube. **1–6:** Inhalation ports. (B) Schematic Diagram of fluidization chamber. (C) Comparative illustration of three fluidization chambers. (D) Schematic diagram showing attachment of fluidization chamber with the dosing chamber (inhalation chamber). (E) View of the doing chamber from the entry port of nebulizer. The noses of mice opened in the dosing chamber are visible in the figure.

hole was connected to the inhalation chamber through a tube. To observe the effect of fluidization volume on the powder delivery, different types of fluidization chambers were made by altering the volume of centrifuge tubes and position of the delivery hole on the wall of the tube. The specifications of three of the fluidization chambers are mentioned in Table 1 and the designs are shown in Figure 1C.

In vitro *evaluation of the inhalation chamber*

The inhalation chamber was examined either with the fabricated DPI or with the marketed nebulizer to assess its efficiency to deliver uniform dose across the inhalation ports.

Evaluation with DPI apparatus. In this study, drug deposition was assessed using fabricated fluidization chamber and the inhalation chamber. This study was performed to select the best DPI design among the prepared ones to get the best deposition in the inhalation ports of the chamber and to aid *in vivo* animal study protocol design. The inhalation chamber was connected to the powder fluidization chamber (DPI) by an interconnecting tube which was simply a polypropylene-pipette tips cut at suitable length to fit for the purpose. The two junctions of the connecting tube were properly sealed to make the path airtight and prevent leakage of powder

Table 1. Description of various dry powder inhalation (DPI) fluidization apparatus.

Fluidization apparatus no.	Volume of the tube (ml)	Position of the hole
DPI 1	15 ml	10–12 ml
DPI 2	15 ml	5–7 ml
DPI 3	50 ml	12–17 ml

during operation. Fluidization apparatus attached to the inhalation chamber is shown in Figure 1D. Three types of fluidization chambers used in this study are shown in Figure 1C. The inhalation chamber designed here can hold up to six mice.

The powder deposition on six inhalation ports inside the chamber was tested by *in vitro* dosing of a mixture (1:3 w/w ratio) of micronized voriconazole (50 mg) with micronized lactose powder (150 mg). Voriconazole and lactose were mixed by manual blending (trituration) using geometrical mixing process. The openings at the tapered ends of the animal holder tubes in the dosing chamber were plugged with cotton. This should be mentioned here that this is an *in vitro* study and hence no animal was used here for estimation of inhaled dose. The positions of the cotton plugs corresponded to the positions of the animal noses if they were placed inside animal holder and thus the collected powder on the cotton plugs represented the total inhalable amount at the respective ports. Powder mixture (200 mg) was placed in the fluidization apparatus and the pipette teat was pressed continuously to dose the powder in the inhalation chamber. At the end of the study, the cottons from different ports were separately collected and drug content was analyzed by HPLC. The extraction of drug from the cotton was conducted by repeatedly rinsing the cotton with methanol and squeezing it with a clean and sterilized forceps and this process was repeated five times, each time using 1 ml methanol. The volume was made up to 5 ml by adding methanol, filtered through 0.22 μm filter (Milipore) and measured by HPLC after suitable dilution with the mobile phase as described later. The powder remained in the DPI devices and in the dosing chamber was also estimated to determine the mass balance.

Evaluation of the inhalation chamber with nebulized dose. In this study, the drug deposition was evaluated using nebulizer and the inhalation chamber. Voriconazole is a poorly water soluble compound and hence it was solubilized in water using HPβCD as a complexing agent. A 10% (w/v) HPβCD solution was used to solubilize the drug. Voriconazole solution of three different concentrations viz. 1, 2 and 5 mg/ml in 10% HPβCD were used for the study and total volume nebulized in each group was equivalent to 50 mg voriconazole. Three different air pressures i.e. 0.5, 0.8 and 1.0 kg-f/cm^2 were used to nebulize drug-solution (2 mg/ml). The air pressure (0.5 kg-f/cm^2) which resulted higher deposition was used to test other drug solutions with concentrations, 1 and 5 mg/ml. The Salter NebuTech HDN Reusable Nebulizer with polyvinyl chloride connecting tubing (i.d 0.5 cm) was used as per the manufacturer's guideline. The solution to be nebulized was placed in the sample holder of the nebulizer. The compressed air required for nebulization was supplied from a cylinder through a regulator connected to a tube attached with the nebulizer. The delivery port of the nebulizer was directly connected with the dosing chamber through the entry port.

The chamber was evaluated for deposition and distribution of aerosol in the opening ports of the dosing chamber. The animals were not placed inside the tube during this study. The study was conducted by filling absorbent cotton at the opening ports of the dosing chamber at the points where animal nose could be exposed in the dosing chamber. Being absorbent type, the cotton can absorb the aerosol till the points it gets saturated and it simulates the inhalable dose at the different ports. At the end of the study, volume of samples remained in the nebulizer (dead volume) was volumetrically measured. The cottons from different ports were separately collected and analyzed by HPLC to estimate the drug content in individual cotton. The drug remained in the nebulizer and the inhalation chamber was also assessed to determine the mass balance.

Determination of MMAD using cascade impactor. Aerodynamic diameter of a particle controls its deposition in pulmonary tract. Mass median aerodynamic diameter (MMAD) represents aerodynamic diameter below which 50% particles remain. MMAD of all the formulations (from Nebulizer and DPI) was determined using an eight-stage non-viable cascade impactor (Thermo Scientific, Waltham, MA). MMAD and geometric standard deviation (GSD)[18] were determined using a flow rate of 28.3 L/min. For studying the particle size from the nebulizer, the solution was kept in the nebulizer sample holder and dosed in the mouth of the cascade impactor. MMAD and GSD are highly dependent on the nebulizer design and nebulization parameters. On the other hand, MMAD and GSD of powder mixture depends mostly on the particle size distribution (PSD) of the mixture. For determination of MMAD from the customized DPI, the same powder composition was taken inside the fluidization chamber and dosed in the cascade impactor. Rest of the process was same as mentioned above. After the completion of dosing, different plates were collected; enhancement of plate weight due to particle deposition was calculated. MMAD was calculated from the deposition data using MMAD calculator for Anderson apparatus[19]. Respirable fraction (RF) is referred to the percentage of drug having particle size of 5 μm or less. It was calculated from the measured PSD data.

In vivo *drug deposition study with nebulizer and DPI*

Swiss Albino mice of either sex and approximately of same age, weighing 20–25 g, were used for the study. They were housed in polypropylene cage and fed with standard diet and water *ad libitum*. The mice were exposed to alternate cycle of 12 h each of light and darkness. Before each test, the mice were subjected to fasting for 12 h. The study was approved by Jadavpur University Animal Ethics Committee.

The animals were placed inside the tube by opening the cap and faced towards the dosing chamber. The void space of the tube was entirely filled with cotton and the tube was capped immediately to stop the movement of the animal inside the tube. The space (if any) surrounding

the animal's nose in the hole of the dosing chamber plate was filled with cotton to stop the flow of the dosage form in other parts of the box except the dosing chamber. Figure 1E shows the positions of animal noses in the dosing chamber and the rest of the animal body inside the tube.

The experimental parameters, which resulted the best deposition in *in vitro* experiment, were selected for *in vivo* studies. *In vivo* drug deposition of voriconazole in lungs was studied from two sets of experiments. One set comprised dosing of 50 mg of voriconazole mixed (1:3 w/w ratio) with micronized lactose (150 mg) as used in *in vitro* study, using DPI device (Apparatus 2). Second set comprised of nebulizing the same amount of voriconazole solution (in 10% HPβCD) having concentration 2 mg/ml at an air pressure of 0.5 kg-f/cm^2.

After the completion of the dosing, the animals were sacrificed and the lungs were collected for drug content analysis. Lungs were processed by a method modified from a reported one[18]. Lungs were homogenized using tissue homogenizer with addition of normal saline (1:1 w/w). 100 µl of tissue homogenate was taken and 20 µl of ketoconazole solution in acetonitrile (100 µg/ml) was added to it as internal standard. To this mixture, 200 µl of acetonitrile was added. The mixture was kept in vortex mixer for 6 min followed by centrifugation at 10,000 rpm for 4 min. The supernatant was reconstituted in mobile phase and was injected in HPLC for drug content determination. Percent *in vivo* drug deposition was calculated by dividing the drug deposited in lungs tissue by the average drug available for inhalation at the ports of the inhalation chamber as obtained from the *in vitro* studies.

HPLC method used for the study

The analysis of drug was carried out in a Perkin Elmer (Waltham, MA) high performance liquid chromatography system using a validated method. The HPLC system consisted of a Perkin Elmer series 200 pump and a Perkin Elmer Series 200 UV/VIS detector set at wavelength of 256 nm. Chromatographic separation was performed on a Perkin Elmer reverse phase C-8 column (37 × 6 mm, 5 µm). Two different mobile phases were used for analysis of drug content during *in vitro* and *in vivo* studies. For drug content determination in *in vitro* study samples, the mobile phase was made of methanol: 10 mM tetramethyl ethylenediamine buffer (TEMED), pH 6.0 at a ratio of 60:40 (v/v) and flow rate was maintained at 2 ml/min. For analysis of drug content in tissue, the mobile phase was a combination of acetonitrile: 10 mM TEMED buffer, pH 6.0 at a ratio of 60:40 (v/v) and flow rate was maintained at 1 ml/min. The reason behind using two different mobile phases was mainly due to difference in the sample processing method for HPLC analysis. The data generated was analyzed with TC Win (version 6.2.0) software (Perkin Elmer). The drug content was calculated from the calibration curve which was developed as mentioned below.

For the construction of standard calibration curve for tissue analysis, 90 µl of lung homogenate was taken in a 1.5 ml centrifuge tube. To this, 10 µl of standard voriconazole stock solution was added followed by addition of 20 µl of 100 µg/ml of ketoconazole solution as internal standard. 200 µl of acetonitrile was added to the mixture followed by vortexing for 6 min and centrifugation for 4 min. The supernatant thus obtained was reconstituted in mobile phase and injected in HPLC. The calibration curve was generated from a concentration range of 0.334–5.25 µg/ml.

Statistical calculations

All statistical calculations were performed with GraphPad Instat version 3.0 (GraphPad Software, Inc., San Diego, CA). The data were analyzed by one-way ANOVA without post test to determine significant differences. Statistical significance was based on probability value of less than 0.05.

Results

In vitro drug deposition study

Evaluation with DPI apparatus

PSD of the micronized voriconazole used for the study was 1.699 µm [d(0.1)], 3.048 µm [d(0.5)] and 5.828 µm [d(0.9)]. PSD of micronized lactose was 2.594 µm [d(0.1)], 5.77 µm [d(0.5)], 11.93 µm [d(0.9)]. Test 1 performed with DPI Apparatus 1 could not fluidize the particle and hence test with this apparatus was not carried further. Test 2 performed with DPI Apparatus 2 was found to deposit an average of 4.5–5 mg (Figure 2A) of voriconazole across the six ports of the inhalation chamber. Test 3 with DPI Apparatus 3 (total volume of 50 ml) was performed to understand the implication of enhancement in the fluidization chamber volume. The results (Figure 2A) show that average deposition across the six inhalation ports was 3–3.8 mg and the mean deposition of all the ports is significantly ($p<0.05$) higher than the mean deposition obtained from Apparatus 2. However, when depositions at individual ports were compared between DPI 2 and DPI 3, depositions were higher across all the ports from DPI 2 but only significantly ($p<0.05$) higher in ports 4 and 5. In the other ports, differences were not significant ($p>0.05$). Mass balance during DPI-use has been included in Table 2A. Amount lost during DPI-use was higher for DPI 3 in comparison to DPI 2.

In vitro *deposition of voriconazole from voriconazole solution using nebulizer*

Effect of various operating parameters on *in vitro* deposition of voriconazole at different ports of the inhalation chamber was evaluated in this study. The drug deposition was studied with solutions having different concentrations (Figure 2B). Three different concentrations, namely 1, 2 and 5 mg/ml, were tested at a fixed air pressure, 0.5 kg-f/cm^2. Drug solution with a concentration of 2 mg/ml resulted highest amount of drug deposition of ~2.0 mg

followed by 1.5 mg and 1.0 mg for drug solutions, 5 mg/ml and 1 mg/ml, respectively. The average drug depositions from the different drug solutions were significantly ($p<0.05$) different from each other across the six ports.

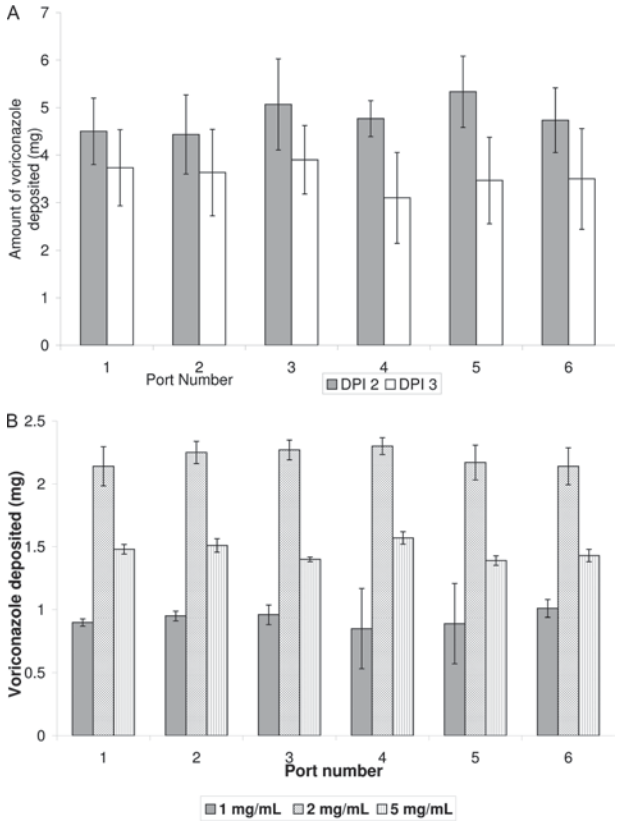

Figure 2. (A) Amount of drug deposited at different ports of the inhalation chamber using two different types of DPI (data as mean ± SD; $n=3$). (B) Effect of solution concentration on drug deposition at different ports of inhalation chamber from the nebulizer (data as mean ± SD; $n=3$). (C) Effect of air pressure used in the nebulizer on drug deposition at different ports of inhalation chamber (data as mean ± SD; $n=3$).

Figure 2C shows drug deposition with variable air pressures during nebulization. The study was conducted at three different air pressures, namely, 0.5, 0.8 and 1.0 kg-f/cm^2 at a fixed solution concentration of 2 mg/ml. The results show that the deposition was nearly uniform in all the six inhalation ports for every set of experiment conducted. A mean range of drug deposition of 2.0–2.25 mg was recorded with 0.5 kg-f/cm^2 air pressure. The mean drug deposition values were found to be about 1.25 mg and 1.0 mg for air pressures 0.8 kg-f/cm^2 and 1.0 kg-f/cm^2, respectively. Results of the experiment at each air pressure shows that variation in drug deposition among six different ports was statistically not significant ($p>0.05$) from each other. However, statistically significant variation ($p<0.05$) was found when drug depositions were compared at the different air pressures studied. Dead volume remained in the nebulizer and mass balance are mentioned in Table 2B. Data show that there was little difference in dead volume remained in the nebulizer at different air pressures at different concentrations. However, the drug content corresponding to the dead volume varied with concentration of the drug solutions.

Determination of MMAD and GSD of various samples

The MMAD and GSD obtained for various samples are shown in Table 3. MMAD (±GSD) of the nebulized droplets generated from solutions of three different concentrations at 0.5 kg-f/cm^2 varied from 4.8 ± 1.61 μm to 7.3 ± 2.65 μm. By increasing air pressure to 0.8 kg-f/cm^2, MMAD was reduced to a minimum of 4.1 ± 1.25 μm to a maximum of 6.2 ± 2.23 μm and enhancement of air pressure to 1.0 kg-f/cm^2 reduced MMAD (±GSD) further to between 3.4 ± 1.19 μm to 5.8 ± 2.11 μm for different solution concentrations studied. MMAD (±GSD) obtained from DPI 2 and DPI 3 was found to be 4.6 ± 1.52 μm and 5.3 ± 1.86, respectively. Depending on MMAD, RF varied widely from 34 to 73% in case of nebulizer and from 47 to 54% in case of DPI as mentioned in Table 3.

Table 2. Mass balance during use of DPI and nebulizer.

A: Mass balance during DPI use							
DPI design		Total dose dispensed (mg)	Amount collected in delivery ports (mg)	Percentage deposition (%)	Amount remained in DPI (mg)	Amount collected in dosing chamber (mg)	Amount lost during dosing (mg)
DPI 2		50	28.83	57.67	5.64	11.23	4.29
DPI 3		50	21.33	42.67	9.23	8.35	11.09
B: Mass balance during nebulizer use							
Nebulization condition	Concentration (mg/ml)	2	2	2	1	2	5
	Air pressure kg-f/cm^2	0.5	0.8	1	0.5	0.5	0.5
Dose dispensed (mg)		50	50	50	50	50	50
Deposited at inhalation ports (mg)		13.27	7.25	6.55	5.56	13.27	8.78
Percentage deposition (%)		26.54	14.5	13.1	11.12	26.54	17.56
Deposited at dosing chamber (mg)		6.32	4.55	3.23	3.58	6.32	5.64
Dead volume in nebulizer (ml)		1.25	1.35	1.18	1.25	1.35	1.46
Amount corresponds to dead volume (mg)		2.5	2.7	2.36	1.25	2.7	7.3
Amount lost during dosing (mg)		27.91	35.5	37.86	34.05	14.44	19.5

Table 3. Mass median aerodynamic diameter (MMAD) of different aerosol formulations.

Device	Air pressure (kg-f/cm^2)	MMAD (μm) at different Solution concentration (with data as mean ± GSD) along with the respirable fraction (RF) value inside bracket		
		1 (mg/ml)	2 (mg/ml)	5 (mg/ml)
Nebulizer	0.5	4.8 ± 1.61 (52.10%)	5.2 ± 1.72 (45.45%)	7.3 ± 2.65 (34.25%)
	0.8	4.1 ± 1.25 (60.97%)	4.5 ± 1.43 (55.55%)	6.2 ± 2.23 (40.32%)
	1.0	3.4 ± 1.19 (73.53%)	3.9 ± 1.34 (64.12%)	5.8 ± 2.11(43.10%)
Device	MMAD (μm) (data as mean ± GSD) along with the respirable fraction (RF) value inside bracket			
DPI 2	4.6 ± 1.52 (54.34%)			
DPI 3	5.3 ± 1.86 (47.17%)			

In vivo drug deposition

The drug deposition in lungs of the animals from both DPI device and nebulizer at six different ports of the chambers is shown in Figure 3. The drug deposition is plotted as voriconazole deposited per unit weight of lung tissue (μg/g) versus inhalation ports. The figure shows drug content at initial time point only. The average initial drug depositions from DPI and nebulizer were found to vary insignificantly ($p>0.05$) and the values were 80–130 μg/g and 40–68 μg/g across the six different ports, respectively. Average drug diposition from DPI was significantly ($p<0.05$) higher than that obtained with the nebulizer. Thus, *in vivo* drug deposition varied from 1.67 to 2.74% w/w of in case of DPI and 1.81 to 3.09% (w/w) in case of nebulizer when the data were compared with those from the *in vitro* drug deposition studies.

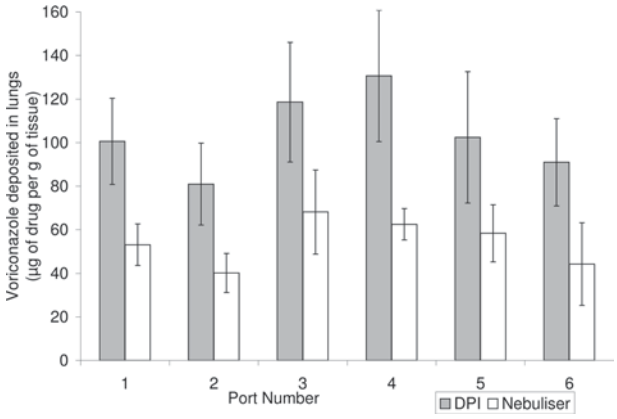

Figure 3. Voriconazole deposition in lungs following DPI and nebulized dose administration of 50 mg equivalent voriconazole (data as mean ± SD; $n=3$).

Discussion

In the present study, a nose-only inhalation chamber was designed using easily available materials. The device can hold up to six mice at a time for pulmonary dosing. Though polypropylene is known to cause electrostatic discharge, the authors did not notice any significant repulsion in presence of dry powder. Polypropylene made tube was earlier used as well by other research group for administration of dry powder to animals[10]. Further, this chamber can be used with either a nebulizer or a DPI. The conventional nebulizer for human-use can be attached with this chamber as shown in Figure 1E. However, the conventional DPIs cannot be used for animals as those require positive inhalation. Therefore, we also designed a DPI that can be attached with the inhalation chamber to dose animals. Initially different designs were tried by variation of the positions of the delivery device and delivery ports. Variation of position of ports was found to alter the total volume of fluidization as well. Ports, at too low position, caused immediate escape of powder. Again, when position of ports was too high, air pressure was insufficient to make the powder escape through the port. The best design out of the prepared ones was reported here. The bigger advantage of this inhalation chamber is that mice can be held inside the tubes with ease; and multiple mice can be dosed at a time. Though the device was used only for mice, but it can be adapted for other small animals by varying the size of the inhalation chamber and animal holding tubes.

The solubility of voriconazole in 10% HPβCD was ~5.5–6.5 mg/ml and marginal improvement of solubility was reported by increasing higher cyclodextrin concentration[20]. Hence the maximum concentration of 5 mg/ml was selected to ensure a stable drug solution for nebulization. Two other lower drug concentrations were tested to observe how drug deposition efficiency varied with the concentrations of the solutions. However, drug concentration was not reduced below 1 mg/ml as lowering drug concentration would increase HPβCD load in comparison to drug. Higher HPβCD load can influence the pharmacokinetics of the drug as well[21]. The MMAD of the nebulized droplets or powder generated from different formulations was found to be between 3.4 ± 1.19 and 7.3 ± 2.65 μm. However, the formulations used for *in vivo* studies had a MMAD of ~5 μm, which is desirable for deep lung penetration. RF of this formulation was found to be 45%. Increasing nebulizer air pressure was found to reduce MMAD due to generation of smaller droplets at higher pressure. Reduction of MMAD was found to increase RF value of the nebulized dose. However, increasing solution concentration enhanced the solution viscosity and reduced the efficiency of the nebulizer to generate smaller droplets and hence MMAD was increased. The DPI 2 had better fluidization capacity due to optimal volume and location of the delivery port. MMAD of the particles obtained by using this apparatus was less than that of DPI 3. However, the variation was found to be significant ($p>0.05$) as the powder composition used.

The total deposition of voriconazole was found to be dependent on the DPI apparatus used. DPI 1 was found to be inefficient to fluidize the powder as effective volume of the fluidization chamber was low due to the position of the delivery port which was very near to the powder bed (Figure 1C). The dispensed powder from the delivery port formed agglomerated particles and most of the powder was out of the fluidization chamber within a short time. Hence, this apparatus was not suitable for further evaluation. Deposition from DPI 2 was significantly improved and cumulative inhalable dose in six different ports reached ~57% of the total dose dispensed from the device. However, further increase of the volume of the fluidization chamber using DPI 3 apparatus reduced the cumulative deposited amount to 42% and it was significantly ($p<0.05$) less than the deposition obtained from DPI 2. Hence, replacement of apparatus 2 with apparatus 3 with a greater fluidization chamber failed to provide better deposition. This might be due to dislodgement of powder from the inhalation port over time using DPI apparatus 3 as it took more time to fluidize powder than DPI 2 because of having more volume.

Drug deposition from the nebulizer was maximum with lowest air pressure (i.e. 0.5 kg-f/cm^2) used and it gradually decreased with the enhancement of air pressure. However, the MMAD reduced gradually with increase of air pressure. Depending on the air pressure, the total drug deposited on cotton varied from a minimum of 13.1% w/w to a maximum of 26.54% w/w of the total powder dispensed. During nebulization, portion of aerosolized solution in the inhalation chamber was deposited in the chamber and most of the part exhausted through the exit port of the chamber. At lower air pressure, volume of air passed per unit time through the nebulizer and subsequently through the inhalation chamber was less. This in turn increased the exposure time for unit volume of sample nebulized. This enhanced exposure time was responsible for lowering the fraction lost and enhanced the total powder deposited. Increasing the air pressure reduced MMAD, but fraction lost was increased due to higher flow rate. As a result, the total amount of powder deposited was reduced.

Drug deposition was found to be maximum when 2 mg/ml voriconazole solution in HPβCD was nebulized at an air pressure of 0.5 kg-f/cm^2 with the nebulizer in the fabricated inhalation chamber. Increasing or decreasing the solution concentration reduced the drug deposition. The minimum drug deposition values were 11.12, 17.56 and 26.54% w/w for drug-solutions with the concentration 1, 5 and 2 mg/ml, respectively (Table 3B). This could be due to increasing solution concentration which might have increased the droplet-numbers per unit volume of nebulized dose and thereby increased drug deposition. However, further increase in solution concentration reduced the total deposition as fraction lost became higher compared to enhancement of drug deposition due to more droplets.

The difference in the drug deposition (μg of drug/g of tissue) obtained in animals in different ports was not significantly different ($p>0.05$) either in case of nebulizer or DPI. The result corroborates the fact that the inhalation chamber along with the fluidization chamber delivered nearly same amount of drug in animals held at the different ports. Though, the total drug deposition was low from nebulizer, but percent drug deposition compared to inhalable dose appeared to be higher in value than that in DPI as inhalable dose from nebulizer was also low. However, the dose available for inhalation and the amount deposited in lungs are comparatively higher than the previous report[10]. The data presented here gives an interesting insight. The maximum total inhalable dose from DPI varied between 40 and 50%. However, the *in vivo* drug deposition depends on other factors as well such as particle aerodynamic diameter, RF and animal breathing pattern[22–24]. Though, in terms of percentage, it looks less, however the amount of deposition was in the range of 50–100 μg/g of lung tissue which is predominantly higher considering pulmonary route of administration. Higher inhalable dose was available from DPI as compared to nebulizer. Further, required exposure time was more in case of nebulizer than DPI.

Conclusions

In vitro study showed that a uniform drug deposition was achieved among various inhalation ports both from nebulizer and DPI. *In vivo* comparative drug deposition study in lungs showed that both nebulizer and DPI device can be used for uniform deposition of drug in lungs across the ports of inhalation in the chamber. The total amount of drug deposited both in *in vitro* and *in vivo* was higher when DPI was used as compared to nebulizer. Again, in the device the dose is available to multiple animals, uniformly among various inhalation ports using nebulizer/DPI. Though, this specific design of inhalation chamber represented here is suitable only for mice, either by altering the number of inhalation ports or altering the dimensions, it could be made suitable to accommodate more number of mice or for other small animals as well. Hence, the inhalation chamber and the DPI device may be used for investigation of pulmonary drug delivery in animal model.

Declaration of interest

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References

1. Watts AB, McConville JT, Williams RO 3rd. (2008). Current therapies and technological advances in aqueous aerosol drug delivery. Drug Dev Ind Pharm, 34:913–922.
2. Adi H,Young PM,Chan H-K, Salama R, Traini D. (2010). Controlled release antibiotics for dry powder lung delivery. Drug Dev Ind Pharm, 36:119–126.
3. Son YJ, McConville JT. (2008). Advancements in dry powder delivery to the lung. Drug Dev Ind Pharm, 34:948–959.
4. Overhoff KA, Clayborough R, Crowley M. (2008). Review of the TAIFUN multidose dry powder inhaler technology. Drug Dev Ind Pharm, 34:960–965.
5. Blagoeva PM, Mircheva TJ, Atanassova RB, Atanassov BT. (1997). Genotoxic changes in the pulmonary alveolar macrophages of mice, rats and hamsters treated with tobacco smoke. J Cancer Res Clin Oncol, 123:253–258.
6. Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR. (1999). Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters. Toxicol Lett, 111:175–187.
7. McConville JT, Williams RO 3rd, Carvalho TC, Iberg AN, Johnston KP, Talbert RL et al. (2005). Design and evaluation of a restraint-free small animal inhalation dosing chamber. Drug Dev Ind Pharm, 31:35–42.
8. Raeburn D, Underwood SL, Villamil ME. (1992). Techniques for drug delivery to the airways, and the assessment of lung function in animal models. J Pharmacol Toxicol Methods, 27:143–159.
9. Negishi T, Nishimura I. (1993). Dust inhalation system for small laboratory animals. Jikken Dobutsu, 42:159–168.
10. Kaur J, Muttil P, Verma RK, Kumar K, Yadav AB, Sharma R et al. (2008). A hand-held apparatus for "nose-only" exposure of mice to inhalable microparticles as a dry powder inhalation targeting lung and airway macrophages. Eur J Pharm Sci, 34:56–65.
11. Nadithe V, Rahamatalla M, Finlay WH, Mercer JR, Samuel J. (2003). Evaluation of nose-only aerosol inhalation chamber and comparison of experimental results with mathematical simulation of aerosol deposition in mouse lungs. J Pharm Sci, 92:1066–1076.
12. Bivas-Benita M, Zwier R, Junginger HE, Borchard G. (2005). Non-invasive pulmonary aerosol delivery in mice by the endotracheal route. Eur J Pharm Biopharm, 61:214–218.
13. Codrons V, Vanderbist F, Verbeeck RK, Arras M, Lison D, Préat V et al. (2003). Systemic delivery of parathyroid hormone (1-34) using inhalation dry powders in rats. J Pharm Sci, 92:938–950.
14. Warheit DB, Brock WJ, Lee KP, Webb TR, Reed KL. (2005). Comparative pulmonary toxicity inhalation and instillation studies with different TiO_2 particle formulations: impact of surface treatments on particle toxicity. Toxicol Sci, 88:514–524.
15. Warheit DB, Webb TR, Reed KL, Hansen JF, Kennedy GL Jr. (2003). Four-week inhalation toxicity study in rats with nylon respirable fibers: rapid lung clearance. Toxicology, 192:189–210.
16. Sumner SC, Asgharian B, Moore TA, Parkinson HD, Bobbitt CM, Fennell TR. (2003). Characterization of metabolites and disposition of tertiary amyl methyl ether in male F344 rats following inhalation exposure. J Appl Toxicol, 23:411–417.
17. Cannon WC, Blanton EF, McDonald KE. (1983). The flow-past chamber: an improved nose-only exposure system for rodents. Am Ind Hyg Assoc J, 44:923–928.
18. McConville JT, Overhoff KA, Sinswat P, Vaughn JM, Frei BL, Burgess DS et al. (2006). Targeted high lung concentrations of itraconazole using nebulized dispersions in a murine model. Pharm Res, 23:901–911.
19. Holt J. 2010. MMAD & GSD Calculator for Andersen Cascade Impactors (ACI) [Online]. Available: http://www.mmadcalculator.com/andersen-impactor-mmad.html [Accessed 1st Sep 2010].
20. Buchanan CM,Buchanan NL,Edgar KJ & Ramsey MG. (2007). Solubilty and dissolution studies of antifungal drug: hydroxybutenyl-beta-cyclodextrin complexes. Cellulose, 14:35–47.
21. Tolman JA, Williams RO. (2010). Advances in the pulmonary delivery of poorly water-soluble drugs: influence of solubilization on pharmacokinetic properties. Drug Dev. Ind. Pharm., 36:1–30.
22. Thompson PJ. (1998). Drug delivery to the small airways. Am J Respir Crit Care Med, 157:S199–S202.
23. Ren Y, Yu C, Meng K, Tang X. (2008). Influence of formulation and preparation process on ambroxol hydrochloride dry powder inhalation characteristics and aerosolization properties. Drug Dev Ind Pharm, 34:984–991.
24. Boshhiha AM, Urbanetz NA. (2009). Influence of carrier surface fines on dry powder inhalation formulations. Drug Dev Ind Pharm, 35:904–916.