Development of novel efficient rodent nose-only inhalation exposure systems

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Summary

It was decided within AstraZeneca R&D to develop novel efficient passive nose-only inhalation exposure systems to enable inhalation administration to rodents in early preclinical development phase. It was decided from the start, when a first novel inhalation system was developed, that it would be a nose-only inhalation exposure system to be used with commercially available restraining tubes. The most important design feature of the novel inhalation exposure system was to develop a closed exposure chamber instead of a flow-pass system in order to improve the utilization of the drug compound. The limited amount of test material available in this early development phase together with practical limitations regarding time and resources for formulation development precluded the possibility to use dry powder aerosols and nebulization was therefore selected as the aerosol generation technique for the novel inhalation exposure system. It was decided to use a vibrating mesh nebulizer with the first novel exposure system. The in-vitro investigations made with a first real prototype system was successful and it was therefore decided to manufacture two fully equipped exposure units for in-vivo use. The first novel inhalation system did unfortunately not meet the expected improvement in utilization of drug compound in in-vivo use. Two other new novel inhalation systems were therefore developed at AstraZeneca R&D. The two new systems have been shown to be significantly more efficient than the first system and also proven to be able to replace intratracheal instillation in the early development phase at AstraZeneca R&D.

Introduction

It was decided within AstraZeneca R&D to develop novel efficient passive nose-only inhalation exposure systems to enable inhalation administration to rodents in early preclinical development phase when new compounds are continuously synthesized and tested to rank compounds in order to identify a lead structure or optimize a lead compound. A direct administration method, intratracheal instillation, was used instead because no commercially available passive inhalation exposure system existed that could deliver appropriate lung doses with the limited amount of test material available in this early preclinical phase at AstraZeneca R&D.

Concerns regarding intratracheal instillation

There are a number of concerns regarding intratracheal instillation. It is invasive delivery of a large amount of vehicle as well as it is applying a dose rate substantially greater than that which would have occurred during passive inhalation and it poses the risk of overwhelming lung defences and causing effects that are not relevant [1]. Other problems are that the intratracheal intubation may cause local irritation and that an unknown amount of the drug may be coughed up or swallowed [2]. Hatch et al showed that mice retained 70% of the instilled radiolabeled albumin in the lungs while the head and carcass (mainly the stomach) contained the other 30% [3]. These figures are in agreement with a similar study in rats performed at AstraZeneca R&D. Perhaps the most consistently reported disparity between passive inhalation and intratracheal instillation relates to the distribution of the deposited dose. Passive inhalation results in a homogenous distribution throughout the lungs, whereas intratracheal instillation generally results in less homogeneity of the dose distribution in the alveolar region and can result in focally high doses of material [1]. Zecchi et al showed in an imaging study that the deposited dose was more concentrated around central airways in intratracheal instillation in comparison with passive inhalation, in which the dose was more uniformly distributed among all the lung sections, reaching also parenchymal regions [4]. A study performed at AstraZeneca where rats were administered a blue dye either via passive inhalation, where the aerosol was generated via nebulisation or via intratracheal instillation also showed that the passive inhalation generated a uniform lung deposition but this was not the case for the intratracheal instillation, see figure 1. The lung was uniformly coloured light blue by the dye after passive inhalation whereas the dye was deposited centrally and patchy after intratracheal instillation.

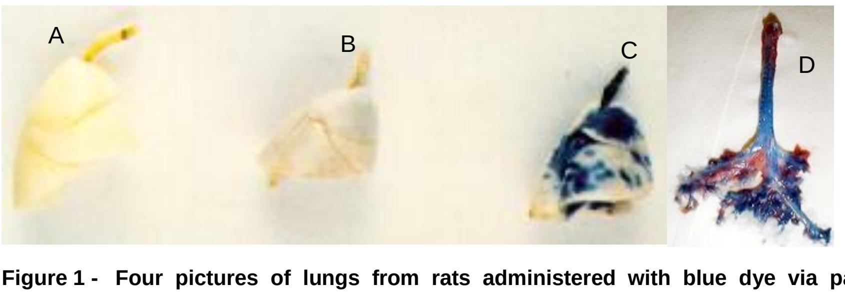

Figure 1 - Four pictures of lungs from rats administered with blue dye via passive inhalation and intratracheal instillation. A = control (no blue dye administered), B = passive inhalation, C = intratracheal instillation, D = dissected central airways from an intratracheal instilled lung

A study to compare the effect of budesonide on sephadex induced lung edema in rats after intratracheal instillation compared with passive inhalation was performed at AstraZeneca R&D. The results showed that the potency of budesonide decreased approximately a tenfold when administered via intracheal instillation in comparison with passive inhalation.

Design of the first novel exposure chamber

It was decided from the start, when a first novel rodent inhalation system was developed, that it would be a nose-only inhalation exposure system to be used with commercially available restraining tubes, see figure 2. A whole-body inhalation exposure system was considered inappropriate because the contamination of the fur when using such a system leads to a very high extent of oral drug administration.

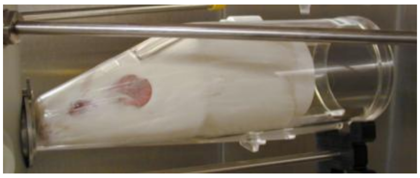

Figure 2 - A rat in a commercially available restraining tube for nose-only inhalation exposure.

Commercially available nose-only rodent inhalation exposure systems are based on the principle of continuously generating aerosol on a carrier air supply and the flow of aerosol is equally divided so that a fraction of the total flow passes in front of the nose of each animal. Each animal will continuously be exposed to freshly generated aerosol. This secures that there is constant quality of the inhaled aerosol but the drug compound will not be utilized efficiently in the system because a major part of the drug will pass through the system without being inhaled. The most important design feature of the novel inhalation exposure system was therefore to develop a closed exposure chamber instead of a flow-pass system in order to improve the utilization of the drug compound.

Other design features that were decided for the first novel inhalation system was that ten restraining tubes would be connected to the closed exposure chamber, that the restraining tubes would be positioned at the same level from the floor of the chamber and that a impeller be positioned in the bottom of the chamber. The impeller would decrease the settling velocity of the aerosol by creating a lifting airflow and thereby increase the residence time of the aerosol in the chamber. The geometrical design of the exposure chamber was first evaluated using computational fluid dynamic simulations on a few different theoretical models, see figure 3.

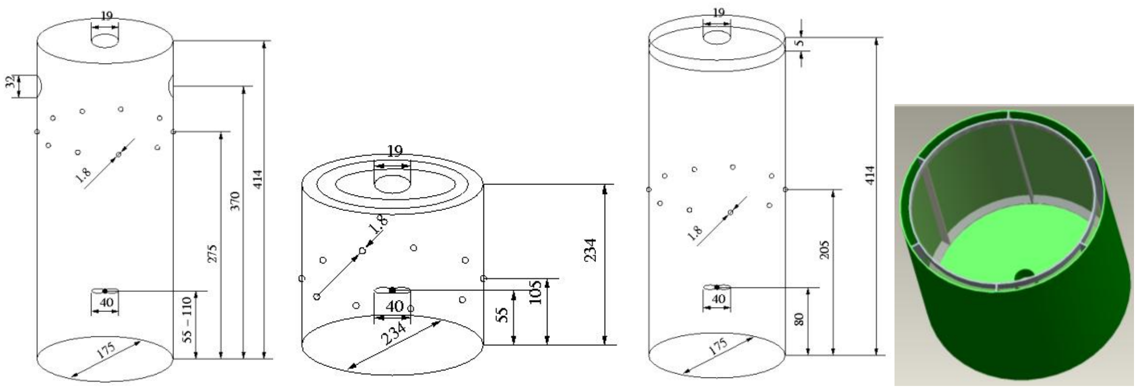

Figure 3 - Three different theoretical models and a plot of the hollow walls of the closed exposure chamber that were evaluated using computational fluid dynamic simulations.

One of the theoretical models was selected and a first real prototype system was manufactured for in-vitro investigations.

It was necessary to solve the problem of keeping an acceptable atmosphere in the closed chamber, i.e. keeping acceptable levels of oxygen and carbon dioxide. One way to solve this issue would be to use pure oxygen in the closed exposure chamber initially and combine this with a short enough administration time. This solution was used successfully by Wu et al when a radioactive aerosol generation and inhalation system was developed and used for an imaging study [5]. Another method was however selected for the design of the first closed exposure chamber system developed by AstraZeneca and that was to use soda lime as a carbon dioxide absorbent that would preclude a build-up of high carbon dioxide levels. Ten rats results in an influx of approximately 60 ml of carbon dioxide per minute into the closed exposure chamber. It was investigated how much absorbent that was needed to avoid build-up of high levels of carbon dioxide and where in the chamber the adsorbent should be placed. It was concluded that 0.5 to 1 kg of soda lime should be sufficient for administration times up to 30 minutes and it should be available both in the walls as well as on the floor of the exposure chamber.

The system was aimed to be used for an administration time of up to ten minutes per group of dosed animals. A ratio of 2:1 of absorbent in the walls compared with the floor was found to be appropriate. The walls were made hollow with a net on the inner wall in order to hold the soda lime in place, see figure 6.

Vibrating mesh nebulizer used as aerosol generator in the first novel rodent inhalation exposure system

The limited amount of test material available in this early preclinical phase together with practical limitations regarding time and resources for formulation development precluded the possibility to use dry powder aerosols and nebulization was therefore selected as the aerosol generation technique for the novel exposure system. The fact that it was a closed chamber system precluded the use of a jet nebulizer because it delivers a volume flow of aerosol, i.e. droplets in a flow of air. It was therefore necessary to use either a vibrating mesh nebulizer or an ultrasonic nebulizer. It was decided to use a vibrating mesh nebulizer, the e-Motion from PARI GmbH. The output rate from this nebulizer was one of the highest from any commercially available nebulizer and it was desired to minimize the exposure time and thus obtain a high aerosol concentration in the chamber as fast as possible. The residual volume in this nebulizer was minimal and this was beneficial from a utilization of drug compound perspective. The vibrating mesh nebulizer generates the aerosol through the pumping action of a perforated plate that vibrates at a high frequency. The plate contains more than a thousand precision-formed holes, surrounded by a vibrating piezoelectric element, which makes the plate vibrate at a high frequency. During each vibration the plate is displaced about one micrometer and it will act as a micro pump, drawing liquid through the holes to form micron sized droplets [6].

A Computational Fluid Dynamic (CFD) investigation was made in order to get a better understanding of the aerosol generation from the vibrating mesh nebulizer into the closed chamber. In order to be able to compare the CFD simulated aerosol generation with a real aerosol generation a vibrating mesh nebulizer was connected to the top of a transparent holding chamber with on open outlet in the bottom and this system was studied both theoretically and visually.

Due to the complex nature of the aerosol generation from the vibrating mesh nebulizer the focus of the study was to get a basic understanding of the physics of the system. There are a lot of changes in the flow characteristics over very small time scales when the aerosol is generated and it was therefore difficult to study the spray with the naked eye and the aerosol was therefore studied with the aid of a high speed camera. A rounded plume and tumultuous swirls could be observed. It was concluded that the droplets from the nebulizer entrained the air, which induced a flow that propelled the droplets beyond the calculated individual stopping distances i.e. there was a two-way momentum coupling between the droplets and the air. The entrainment of the initially stagnant air was so extensive that turbulence was generated in the air and a turbulence model was needed to model the system. When modelling the droplets sprayed into the chamber, calculations were made to roughly estimate the amount of evaporation that could occur to saturate the air in the chamber. It could be concluded that a relatively small amount of water was needed to saturate the air and only a small amount of the water sprayed from the nebulizer would therefore evaporate. To simplify the calculations evaporation was not taken into consideration in the simulations. Droplets that hit the walls of the holding chamber were assumed to stick to the walls and wet them. The CFD calculations were evaluated by comparing the simulated results with the visual observations using the high speed camera and it was concluded that there was a good qualitative agreement between the calculated results and the visual observations. The extent of turbulence was estimated, see figure 4. The turbulence was calculated to be generated mostly at the inlet where the spray of droplets was most dense and that it created swirls in the air in the chamber.

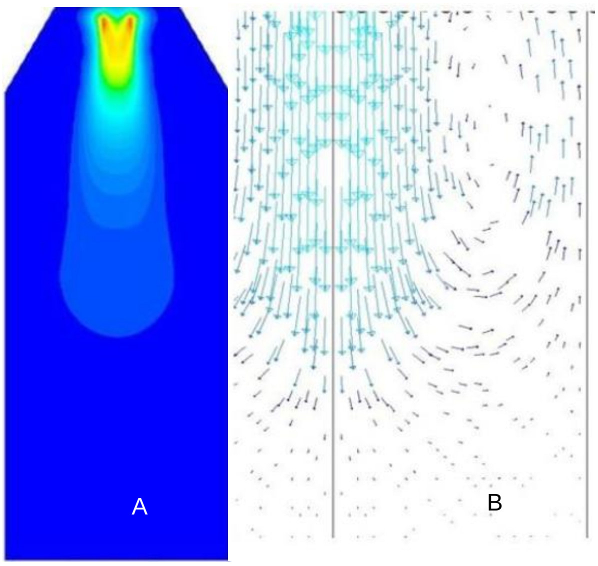

Figure 4 - A. Contour plot of the turbulent kinetic energy in the holding chamber. B. Illustration of swirls in the air velocity vectors

In-vivo evaluation of the first rodent inhalation exposure system

The in-vitro investigations made with the first real exposure system was successful and it was therefore decided to manufacture two fully equipped units for in-vivo use, see figure 5. An in-vivo test of a fully equipped unit was performed using Wistar rats. Mometazone furoate 10 mg/ml nanosuspension was nebulized continuously for three minutes into the chamber. The rats continued to inhale and exhale from the chamber for an additional two minutes after the nebulizer had been stopped, i.e. the total administration time was five minutes per group of animals. The rats were then dismounted and sacrificed immediately, by an intraperitoneal injection of pentobarbital sodium. The lungs were dissected and homogenized. The substance concentration was determined by LC-MS-MS. The amount of Mometazone suspension nebulized, was determined by weighing the nebulizer before and after each test-run. The result was that 0.4 to 1.1% of the nebulized amount of drug deposited in the lungs of the rats. This was significantly lower than expected from the in-vitro investigations of the system.

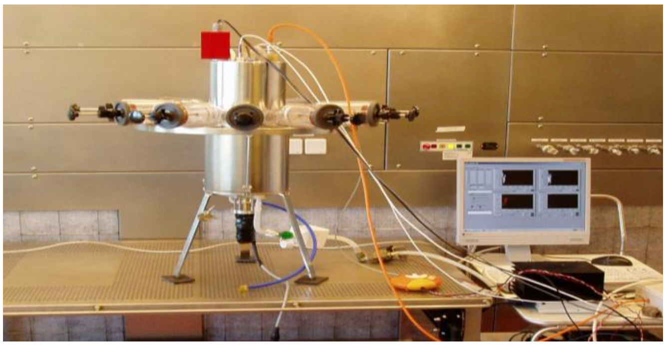

Figure 5 - A first novel functional fully equipped nose-only aerosol exposure system for in-vivo use

Further development – redesign of the novel rodent inhalation exposure system

The first novel rodent inhalation exposure system did not meet the expected improvement in utilization of drug compound in in-vivo use and was therefore not a suitable replacement for intratracheal instillation in the early development phase.

The exposure system was therefore thoroughly redesigned and two new novel closed systems with significantly different designs were developed. An individual rodent system was developed and initially used to administer a radioactive tracer in a study of mucociliary clearance using SPECT imaging. A four-rodent system was also developed and it was initially used for pharmacokinetic studies. Other uses of these two systems will be tested and evaluated. The most important design differences was that the new systems were significant smaller, lacked an impeller to increase the aerosol residence time and also lacked carbon dioxide absorbent that precluded a build-up of high carbon dioxide levels. A vibrating mesh nebulizer, Aeroneb Lab from Aerogen, was used initially with both of the two new systems, but also a jet nebulizer, Cirrus2 from Intersurgical Ltd., has been used successfully with both systems and the use of an ultrasonic nebulizer is currently being evaluated for use. The two new systems have been thoroughly tested and evaluated in both in-vitro and in-vivo studies and they have been shown to be significantly more efficient than the first exposure system and also proven to be able to replace intratracheal instillation in early development phase at AstraZeneca R&D.

References

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