Dry powder inhalation of antibiotics
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In vitro evaluation of the DP-4M PennCentury™ insufflator

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Abstract

Dry powder formulations for inhalation have to be screened in animal studies for therapeutic efficacy and safety aspects and both are significantly affected by the dose and the particle size distribution (PSD) of the aerosol that is given. One of the most frequently used apparatus for pulmonary delivery of dry powder formulations in mice studies is the PennCentury™ DP-4M Dry Powder Insufflator. To make researchers of future preclinical animal studies with the DP-4M insufflator aware of the pitfalls regarding the conclusions to be drawn from their data, we investigated the dispersion behaviour by the DP-4M insufflator using two to three different powder preparation techniques for four different compounds. The primary PSDs of the different formulations were determined in duplicate by laser diffraction analysis. To measure the PSDs of the aerosols obtained with the DP-4M insufflator, the same diffractometer was used in combination with an in-house constructed adapter for the insufflator. The dispersion efficiency and delivered dose were highly affected by the amount of air available for dispersion; the 200 µl of air recommended for the type of insufflator used was insufficient for adequate dispersion. In contrast, the weighed dose did not have a profound effect on the dispersion behaviour and the delivered dose of the DP-4M insufflator. Also the physico-chemical powder properties and the applied particle preparation technique influenced the amount and PSD of the delivered aerosol only to a limited extend, with a few exceptions. We advise researchers to investigate the dispersion efficiency and delivered dose from the DP-4M insufflator with the formulation under investigation prior to in vivo studies and it may be necessary to optimise the formulation for administration to mice.
Introduction

There is a growing interest in the pulmonary route for the administration of drugs currently being delivered via the parenteral or oral route [1, 2]. This includes therapies against local diseases that start and flourish predominantly in the lungs, such as tuberculosis and influenza, for which delivery to the respiratory tract is delivered directly to the site of infection [3]. Pulmonary administration of for instance antibiotics may result in higher local concentrations, lower total doses and less systemic side effects [2]. The administration of vaccines (such as influenza or measles) to the respiratory system has already shown to be effective [4]. Also for systemically acting drugs, the respiratory tract may be an interesting port of entry [5, 6]. By inhalation, first-pass effects in the gastrointestinal tract and liver are avoided which increases the bioavailability of for example small peptides and proteins compared to oral administration [2].

The feasibility testing of pulmonary drug administration starts with preparing suitable formulations for nebulisation, pressurised metered dose inhalers or dry powder dispersion. Such formulations have to be screened for therapeutic efficacy and safety aspects that are significantly affected by the efficacy of the administration which for pulmonary administration correlates with parameters such as dose and the particle size distribution (PSD) of the aerosol that is given [7, 8]. Particularly for systemically acting drugs the site of deposition may be extremely important as most larger molecules can only be absorbed effectively via the most distal part of the airways [9, 10]. Targeting of the lower respiratory tract for such drugs is therefore mandatory and this requires good correlation between the PSD of the aerosol and the flow rate with which the aerosol enters the oral cavity. Testing of pulmonary drug efficacy mostly starts in small rodents such as mice and rats in spite of the fact that small animals have a completely different lung morphologies and geometries compared to humans [11-13]. Intratracheal instillation is frequently used for such experiments and it is assumed that the total lung is targeted with this technique. However, instillation does not take account of the possible effect of the aerosol properties on the systemic or local drug efficacy. Therefore, alternative methods are reported in the literature of which insufflation with a wet or dry aerosol generator seems to approach inhalation best [14-17]. Different principles for insufflation of wet and dry aerosols have been developed for different animal types [17]. One of the most frequently used apparatus is the PennCentury™ DP-4M Dry Powder Insufflator for mice [4, 18, 19].
Although many studies with the DP-4M insufflator provide valuable information about the drug efficacy and even seem to enable a rough estimation of the dose needed for humans, they also may leave many questions unanswered. For instance, frequently the PSD of the aerosol administered with the insufflator is not known. This increases the uncertainty about the aerosol penetration depth into the lungs and the drug distribution over the lung tissue, which are parameters known to influence the drug efficacy significantly. A notorious miscomprehension in this respect is the assumption that the primary PSD of the powder (as for example measured with laser diffraction technique using a highly efficient RODOS disperser) is the same as the size distribution of the aerosol generated by the insufflator [20]. Obviously, not knowing the quality of the delivered aerosol could result in incorrect data interpretation for studies in which these insufflators are used. At least the uncertainties mentioned make drawing conclusions about the preferred site of deposition impossible and direct extrapolation towards the dose needed for humans arguable. Also the efficacies of the insufflator and the future inhalation device with respect to powder dispersion may be completely different. This may result in different delivered fine particle doses with different properties, which has the consequences of different total lung doses and different drug distributions over the lung between both delivery devices. Knowing more details in this respect increases the predictive value of insufflator studies and it is the objective of this manuscript to address these aspects.

We investigated the dispersion behaviour from the PennCentury™ insufflator of two to three physical variants of four different compounds to make researchers of future preclinical animal studies with the DP-4M insufflator aware of potential pitfalls regarding the conclusions to be drawn from their data. We selected two antibiotics and two excipients that are frequently used for the stabilisation of vaccines and biopharmaceuticals as model compounds. We investigated the effect of dose weight and volume of the air pulse on the dispersion behaviour and delivered dose with the PennCentury™ DP-4M Dry Powder Insufflator to show the significant differences that may occur in the PSDs of the aerosols amongst each other and compared to the primary PSDs of the powders as measured with laser diffraction technique from RODOS dispersion. Furthermore, the variations in delivered dose were investigated.
Materials and methods

Materials

Colistimethate sodium (CMS) was purchased from Xellia Pharmaceuticals ApS (Denmark), tobramycin as free base (TOB) from BUFA (the Netherlands), inulin (INU) with a degree of polymerisation of 23 from Sensus (the Netherlands) and mannitol (MANN) from Sigma-Aldrich (Germany).

Four DP-4M Dry Powder Insufflators, an AP-1 air pump and a 3 mL syringe were purchased from PennCentury™ (USA).

Methods

Powder preparation techniques

Three different powder preparation techniques, commonly used to achieve the desired aerodynamic PSD for inhalation, were applied to investigate their effect on delivery with the DP-4M Dry Powder Insufflator. All four model compounds were micronised with a 50 AS spiral jet mill (Alpine, Germany) operated at a nozzle pressure of 6 bar using a 0.8 mm nozzle and a milling pressure of 2 bar. The milling gas was water free nitrogen which enabled to prevent moisture uptake by the powders during size reduction. Spray drying of all four model compounds was performed with a Mini Spray Dryer B-290 (Büchi Labortechnik AG, Switzerland). The inlet air temperature was set to 120 °C, the aspirator to 100%, the pump speed to 8%, and atomizing airflow rate to 50 mm. The concentration of the spray dried solutions was the same for all materials: 50 mg/mL. Spray freeze-dried formulations were only prepared for MANN and INU, since these materials are frequently used as excipients or matrix formers in powder formulations containing biopharmaceuticals prepared with the same drying technique [21]. The solutions were sprayed through a 0.7 mm two-fluid nozzle, with an atomizing airflow of approximately 500 L noting/h, directly into liquid nitrogen to rapidly freeze the droplets which were next vacuum dried in a Christ model Epsilon 2–4 lyophiliser (Salm en Kipp, The Netherlands). First the temperature of the samples was equilibrated on a pre-cooled shelf (-50 °C) for 1 h. Next, primary drying was performed at -35 °C and the pressure decreased to 0.220 mbar. During secondary drying the temperature was gradually increased to 20 °C at a pressure of 0.05 mbar.
Laser diffraction measurements

The PSDs of the different formulations were determined in duplicate by laser diffraction analysis (LDA) after RODOS dispersion at 1, 3 and 5 bar using a HELOS BR laser diffractometer (Sympatec, Germany). To determine the PSDs of the aerosols obtained with the insufflator, using the same diffractometer, the DP-4M insufflator was mounted on an in-house constructed mounting plate to keep the tip of its capillary at a constant distance from the laser beam (Figure 2.1). The dispersion efficiency was determined in triplicate for all experiments and the DP-4M insufflator connected to the AP-1 air pump or a 3 mL syringe was fired four times maximally per dose. Appropriate trigger conditions were adjusted to start the measurement with a 100 mm (R3) lens (measuring range 0.45–175 µm) at the moment the aerosol passed the laser beam and the PSDs were calculated based on the Fraunhofer theory.
Parameters varied during dispersion experiments

Next to different model compounds and particle preparation techniques, two additional experimental parameters were varied to evaluate the dispersion efficiency of the insufflator. The amount of air for dispersion was initially set to the recommended 200 µL for the DP-4M, but to investigate whether dispersion depends on the air volume, also 500 and 1000 µL were tested. 200 µL was generated with a calibrated AP-1 air pump and 500 and 1000 µL were generated with a 3 mL syringe. Furthermore, three different dose weights were investigated. Based on the information provided by PennCentury™ (recommending a dose between 1–2 mg), the study was started with 1 mg, except for spray freeze-dried INU which appeared to be too voluminous to fit in the dose compartment. For this reason and because doses lower than 1 mg are described in the literature [19], 0.5 mg doses of spray freeze-dried powders (INU and MANN) were tested and for comparison, the micronised and spray dried samples of these compounds were dispersed at the same dose weight. Because in the literature also higher doses are mentioned [18], tests were completed with 3 mg doses (only for the micronised and spray dried powders). All doses were carefully weighed into the dosing chamber of the DP-4M insufflator using a Mettler Toledo XP105 Delta Range Analytical Balance (Mettler-Toledo Inc., USA).

Measurement of the delivered doses from the DP-4M insufflator

The amount of powder retained in the DP-4M insufflator was determined after 1 to 4 pulses of air, depending on the number of pulses for which a PSD could be measured accurately with the laser diffractometer. In combination with the dose weight measured into the insufflator this enabled calculation of the delivered dose. Measuring was carried out gravimetrically for the doses of 1 mg and higher of all four compounds and by chemical analysis for the 0.5 mg doses (only applied for MANN and INU). For gravimetical analysis the same Mettler Toledo XP105 Delta Range Analytical Balance was used as for weighing of the doses. To chemically quantify the MANN retention the Hantzsch-reaction followed by UV spectrophotometry at a wavelength of 420 nm and for INU the anthrone-test followed by VIS spectrophotometry at a wavelength of 630 nm were applied [22, 23]. A UV-VIS Unicam UV 500 spectrofotometer (Gemini BV, the Netherlands) was used for the analyses.
Results

Primary particle size distributions of the powders

Figure 2.2 shows that the PSDs of the powders obtained from LDA differ between the different compounds using the same preparation technique and conditions. As to be expected, they also differ between different preparation techniques for the same compound. Most micronised formulations are finer than the spray dried formulations, except for CMS. Figure 2.2 furthermore shows that most PSDs are more or less independent of the dispersion pressure, except for the spray freeze-dried formulations. It is assumed that the results obtained at 3–5 bar represent the primary PSDs and the volume median diameters ($X_{50}$-values) obtained at these pressures are fairly well within the desired range for inhalation (1–3 micron).

![Figure 2.2 PSDs of micronised (Micro), spray dried (SD) and spray-freeze dried (SFD) CMS, TOB, INU and MANN determined by LDA using a RODOS dry powder disperser operated at 1, 3 and 5 bar and the HELOS BR laser diffractometer. The Y-axis is limited to 15 µm, therefore some particle diameters are presented in the figure.](image)

Dispersion efficiency of the DP-4M insufflator

As a measure for the dispersion efficiency of the DP-4M insufflator $X_{50}$-values were derived from the PSDs of the aerosols from the insufflator for comparison with the $X_{50}$-values from RODOS dispersion. Figure 2.3 shows that significant differences in dispersion efficiency between the RODOS disperser and the DP-4M insufflator exist for
Figure 2.3  Comparison of the $X_{50}$ values of micronised (Micro) and spray dried (SD) CMS (A) and TOB (C) and micronised, spray dried and spray-freeze dried (SFD) MANN (B) and INU (D). RODOS1/3/5: pressure used in bar. 200/500/1000: volume of air used for dispersion in µL per pulse of air. 0.5/1/3: dose in milligram. The missing bars indicate that the dose did not fit in the dose compartment, so the measurement could not be taken. The error bars indicate the standard deviation from the mean.
all powders. Aerosols produced with the prescribed 200 µL air pulse have high $X_{50}$-values indicating that dispersion with the DP-4M insufflator is rather incomplete using this volume of air. Moreover, dispersion is highly variable when using 200 µL air pulses as can be concluded from the wide ranges for the standard deviation bars. Noticeably, the dose weight dependence is not consistent between the powders. For MANN the effect of dose weight is highest for the micronised powders; for CMS this effect seems more pronounced for the spray dried powders. Generally, but not consistently, spray dried powders dispersed better than micronised powders with 200 µL pulses of air. The differences in results from different dose weights and air volumes for different powder types suggest that general trends cannot be given. The much better dispersion with 500 µL and 1000 µL air pulses for most powders at most dose weights indicates that a volume of 200 µl is insufficient to operate the DP-4M insufflator adequately. However, even with these higher air volumes for dispersion, the primary PSDs for the micronised MANN and TOB 3 mg samples were not approached. Overall, MANN powders were dispersed least effectively.

Delivered dose from the DP-4M insufflator

The inhaler retention, determining the delivered dose, appears to be also largely dependent on the amount of air used for dispersion (Figure 2.4) and shows similar to the dispersion efficiency a considerable variation. Using 500 or 1000 µL of air for dispersion generally increases the delivered dose compared with 200 µL for all formulations at all doses investigated, but the effect of air volume on delivered dose is less pronounced than that on the dispersion with an exception for mannitol. The effect of dose weight on delivered dose seems highly inconsistent between the different powders which may be attributed to the relatively large spread between duplicate experiments.

Discussion

The DP-4M Dry Powder Insufflator™ is specifically developed for pulmonary drug administration to mice and variants suitable for rats, guinea pigs and larger animals exist [24]. To investigate the dispersion efficiency of the DP-4M insufflator, four different model compounds from different particle preparation techniques were investigated. CMS was selected as a reference, because we found in earlier work (unpublished data) that micronised CMS is efficiently dispersed with the DP-4M when a sufficient amount of air for dispersion is used. TOB was selected because of its unfavourable physico-chemical properties for
Figure 2.4  Comparison of the retention values of micronised (Micro) and spray dried (SD) CMS (A) and TOB (C) and micronised, spray dried and spray-freeze dried (SFD) MANN (B) and INU (D). RODOS1/3/5: pressure used in bar. 200/500/1000: volume of air used for dispersion in µL per pulse of air. 0.5/1/3: dose in milligram. The missing bars indicate that the dose did not fit in the dose compartment, so the measurement could not be taken. The error bars indicate the standard deviation from the mean.
dispersion in a dry powder inhaler due to its high hygroscopicity and cohesiveness [25]. The polyol MANN was chosen, because it is often used to increase the dispersion efficiency of dry powder formulations for inhalation [18, 26, 27] or to stabilise proteins or vaccines in dry powder formulations. INU is a polysaccharide also used to stabilise proteins or vaccines in dry powder formulations [20, 28].

Figure 2.2 shows that the laser diffraction diameters of the spray-freeze dried particles are considerably larger than those of the micronised and spray dried particles. As a result of the rapid droplet cooling in liquid nitrogen as part of the spray-freeze drying technique, the PSD of the frozen droplets is almost the same as the original droplet size from the two-fluid nozzle and the PSD remains the same during sublimation of the solvent too. This results in a highly porous particle structure and much larger particle diameters for the spray freeze-dried powders compared to the spray dried and micronised particles [29]. The lower density and larger diameter of spray freeze-dried particles compensate widely for each other however, and as a consequence of that, the PSDs of these powders differ aerodynamically not to the same extent from the PSDs of the micronised and spray dried powders as they differ optically. Besides, differences between laser diffraction and aerodynamic diameters for the same powder or differences in the aerodynamic diameters between the different powders in this study are not relevant. The aim was to investigate the dispersion efficiency of the powders with the DP-4M insufflator by comparing the PSD in the aerosol with the PSD from RODOS dispersion using the same measuring technique, and for this comparison the type of diameter is irrelevant.

The $X_{50}$-values presented in Figure 2.3 for the aerosols from the insufflator are only for the first 200 µl air pulse. $X_{50}$-values of aerosols produced of the same dose with subsequent air pulses were measured too, but it was decided not to average all (maximally 4) values for the same dose because weighing the insufflators after each air pulse showed that the vast majority of the delivered dose from the insufflator was already released in the first air pulse. This resulted in considerable differences in the optical concentrations between the aerosols of successive measurements of the same dose for which corrections would be needed, to prevent overrating of the size distributions for aerosols with lower optical concentration. In Figure 2.3 (and 2.4) the error bars indicate the standard deviation from the mean. The large error bars show that the difference between experiments in most of the experiments is considerable. This indicates that the performance of the DP-4M insufflator, with the investigated powders, is poorly reproducible. Remarkably TOB, selected for its poor dispersion behaviour in inhalers, dispersed most efficiently with the DP-4M insufflator,
whereas MANN, known from the literature as a compound used to improve the dispersion behaviour [26], dispersed worst.

In many animal studies the PSDs of the powders administered with PennCentury™ insufflators are not known. Instead of measuring the PSDs in the aerosols from the insufflators, highly effective dispersion principles (e.g. RODOS or Aero S) are used [20, 30]. It is then assumed that the PSD from both types of dispersers is the same. Cascade impaction analysis is another technique described in the literature to characterise the aerosol cloud of formulations to be administered with the insufflator, but instead of using the insufflator itself for dispersion of the powder, a standard capsule based inhaler is used [31-34]. From the results presented in Figure 2.3, it can be concluded that RODOS experiments have little predictive value for the aerosol properties from the insufflator. Particularly for the prescribed 200 µL air pulse differences in the PSD between the aerosol from the insufflator and that from RODOS dispersion are extreme for all four powders and all preparation techniques.

Although studies have shown that there is not a significant difference in lung deposition between 1 or 3 µm particles in mice, it is reasonable to assume (based on particle dynamics) that particles with an aerodynamic diameter smaller than 5 micron are needed to effectively penetrate the lung lobes of mice [11-13]. Particles larger than 5 µm, particularly at the high velocity with which they are released from the insufflator, will not be able to travel much deeper into the respiratory tract than the carina. Figure 2.3 shows that particles with a median diameter smaller than 5 µm can in most cases only be obtained with air volumes of 500 and 1000 µL. Only for the lowest doses of spray dried MANN, TOB and INU (0.5 and 1 mg) the X₅₀-values are smaller than, or approach 5 µm with an air volume of 200 µL. Aerosols of micronised and spray-freeze dried powders and the high dose (3 mg) spray dried samples contain significant amounts of agglomerates from the insufflator when operated with a 200 µL air pulse (for which the DP-4M insufflator is actually designed). Although this study shows that the DP-4M insufflator can easily be operated with higher air volumes, and much better results are obtained with these higher volumes, practically they cannot be used for mice, because the maximum air pulse that can safely be used in mice is around 250 µL [19].

Figure 2.4 shows that in many cases the dose weight does not have the same huge effect on the delivered dose as the amount of air used for delivery of the dose. Also the physicochemical powder properties or the technique used to prepare the powders seem of lower
importance than the air volume. These findings make the limitation of the amount of powder between 1 and 2 mg by the manufacturer for the DP-4M insufflator somewhat arguable. On the one hand, 0.5 mg of mannitol or inulin (all preparation techniques) may already be too much for adequate dispersion with the DP-4M (smaller quantities were not tested). In contrast, for micronised colistimethate sodium the PSD in the aerosol does not change significantly when the dose is increased from 1 to 3 mg (200 µL pulses), whereas the retention is more than 50% lower for the higher dose. This suggests that limitation of the dose should not so much be on the basis of weight, but rather on the basis of powder properties. Considering that mice poorly tolerate multiple doses from an insufflator, it may be better to increase the dose (to even more than 2 mg) for a single administration than splitting the dose in smaller portions for multiple administration for powders with good dispersion behaviour. Therefore, it is worthwhile investigating dispersion efficiency before using the insufflator and it may also be helpful to specifically formulate the powders for delivery with the DP-4M insufflator. The retention results in Figure 2.4 make clear that experiments are of lower value when weighing of the insufflator before and after the test to determine the delivered dose (as recommended by the manufacturer) is omitted as this makes it impossible to determine dose-response relationships. Using 500 or 1000 µL pulses of air for dispersion in general increases the dispersion efficiency and delivered dose consistency. This could indicate that larger rodents such as rats and guinea pigs or larger animals like dogs, sheep and monkeys are more appropriate for in vivo toxicology, lung deposition and pharmacokinetic studies of dry powder formulations for inhalation than mice. Mice are often selected for initial studies in pharmaceutical and pharmacological research, because relatively large numbers of these animals can be used for statistical validity [17]. Mice are also cheaper to purchase and easier to house and handle than larger animals. Alternatively, early efficacy and safety studies could be performed with an effective wet nebulisation method such as the MircoSprayer® or by instillation.

So-called nose-only, head-only or whole body inhalation chambers could be another alternative for intratracheal insufflation in mice [17, 35], although a major disadvantage of head only or whole body chambers is the possibility of drug absorption by other routes such as the oral route. Advantages are that no preparative surgical procedures and anaesthetics are necessary. Important differences in inhalation chambers compared to administration with insufflation are that inhalation is with tidal breathing and the aerosol will pass the nasal cavity were most of the deposition will occur, since rodents are obligate nasal breathers. Studies have shown that 80–90% of inhaled aerosols with a PSD between 1 and 5 µm is deposited in the
nasal cavity [11, 12], whereas the nasal cavity is circumvented in intratracheal insufflation, because the formulation is forced directly into the trachea with an air pump. Furthermore, it is difficult to create an aerosol in the desired PSD in an inhalation chamber, and even harder to maintain this aerosol over a period of several minutes.

Conclusions

Our aim was to make researchers of future mouse studies with the DP-4M insufflator aware of the pitfalls regarding the variations in delivered dose and aerosol generation efficiency of this type of insufflator. It must be anticipated that these variations largely affect the conclusions to be drawn from such studies. It was shown for four different model compounds (prepared with different techniques) that the dose weight does not have the most dominant effect on the dispersion behaviour and the delivered dose of the DP-4M insufflator. Also the physico-chemical powder properties and the applied particle preparation technique influence the amount and properties of the delivered aerosol to only a limited extent, with a few exceptions. In contrast, the dispersion efficiency and delivered dose were highly affected by the amount of air available for dispersion. The 200 µl pulses of air recommended for the type of insufflator used appeared to be insufficient for adequate dispersion of all four compounds tested. This conclusion is irrespective of the preparation technique used, although spray dried powders performed in general better than micronised and spray-freeze dried powder in this study. The findings in our study can shade conclusions drawn from the animal studies with regard to the efficacy or safety of dry powder formulations. Therefore, it is advised to investigate the dispersion efficiency and delivered dose from the DP-4M insufflator with the formulation under investigation prior to the in vivo study and it may be necessary to optimise the formulation for administration to mice with the DP-4M insufflator.

References


In vitro evaluation of the DP-4M PennCentury™ insufflator

Chapter 2


