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In vitro and in vivo aspects of quantifying intrapulmonary deposition of a dry powder radioaerosol

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Abstract

Pulmonary delivery of pharmaceutical aerosols can be quantified using gamma scintigraphy. Technetium-99m, the most commonly used radionuclide in scintigraphic studies, cannot be incorporated into the drug molecule and, therefore, may be distributed differently from the drug itself, particularly if the drug is presented as a solid in a liquid suspension or as a dry powder formulation. This study demonstrated the importance of using conditions relevant to the in vivo situation in the in vitro characterisation of a dry powder aerosol of ^{99m}Tc-labelled lactose. The influence of inspiratory flow on the distribution of aerosol within the lungs was investigated in eight healthy subjects who inhaled the ^{99m}Tc-labelled lactose at four flows (30, 40, 60 and 80 l/min). No differences in penetration index (PI) or count density distribution of radioactivity were seen, indicating that regional distribution of aerosol in healthy airways was insensitive to differences in the inspiratory effort exerted by the subject while inhaling the experimental dry powder radioaerosol. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Radionuclide imaging; Gamma scintigraphy; Radioactive aerosol; Administration, inhalation; Technetium 99m; Powder inhaler

1. Introduction

For any device designed to deliver a drug by oral inhalation, the fraction of dose that escapes impaction in the upper airways and penetrates into the lung will depend on the patient's anatomy and mode of inhalation. Scintigraphic as well as pharmacokinetic methods can be used to quantify the total amount of drug reaching the lungs, but to obtain detailed information about the regional distribution of aerosol within the complex lung structures a scintigraphic method has to be used. One such method involves aerosol labelling with the gamma-ray-emitting radionuclide technetium-99m (^{99m}Tc) and planar scintigraphic measurements. The usefulness of this method to quantify pulmonary delivery of a dry powder formulation was evaluated in this study.

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The penetration index (PI), i.e. the ratio of peripheral to central lung zone deposition of radioactivity, can be estimated to obtain an approximation of the regional distribution of inhaled drug within the airways. It has been demonstrated in healthy subjects that slow inhalation of ^{99m}Tc-labelled aerosols delivered by pressurised metered dose inhalers promotes aerosol penetration, i.e. gives a higher PI than a fast inhalation (Newman et al., 1989a, 1995). On the other hand, a moderate change in inspiratory flow does not seem to influence the PI of ^{99m}Tc-labelled aerosols inhaled via dry powder inhalers (Borgström et al., 1994; Newman et al., 1994; Pitcairn et al., 1997).

The primary aim of this study was to determine the influence of a range of inspiratory flows on the regional distribution of a dry powder radioaerosol within the airways. For this purpose, an experimental formulation of ^{99m}Tc-labelled lactose was developed, filled into and delivered via a multi-dose dry powder inhaler, Turbuhaler® (AstraZeneca). Children and adults with airway obstruction normally generate an inspiratory flow through Turbuhaler of about 60 l/min, irrespective of age (above 4 years) or severity of airway disease (Brown et al., 1995; Meijer et al., 1996; Agertoft and Pedersen, 1998; Dewar et al., 1999). Few patients seem unable to generate a flow of 40 l/min or more. However, to adequately validate the scintigraphic method in this study, inspiratory flows within the range of 30–80 l/min were selected in order to include flows below and above those normally generated by patients.

2. Materials and methods

2.1. Radiolabelling method

Radiolabelling was based on a method originally described by Köhler et al. (1988), and developed by Newman et al. for terbutaline sulphate in Turbuhaler (Newman et al., 1989b). The final radioactivity concentration had to be balanced against the maximum amount of radioactivity that could be safely used in the labelling process, the minimum powder fill weight required to ensure reproducible dosing, and minimum count rate needed in the scintigraphic measurements. About 3 GBq of ^{99m}Tc-sodium pertechnetate in 0.5–1.5 ml of isotonic saline was eluted from a ^{99}Mo/^{99m}Tc generator (Ultra-TechneKow® FM, Mallinckrodt Medical BV, Holland). When necessary, the eluate was diluted with water to 1.5 ml. The radioactivity (^{99m}Tc) was extracted twice with 1.5 ml of methyl ethyl ketone (MEK). The extract was placed in a heating block and the organic phase evaporated under a stream of nitrogen until dryness (for about 40 min) at $+45$ °C. The residue was dissolved in 0.2 ml of tertiary butanol and the organic phase evaporated. This procedure was repeated and the resulting residue dissolved in 0.5 ml of extensively dried MEK (dried above a 4A molecular sieve: the MEK was distilled twice with the mid half portion collected and kept above the molecular sieve until use). The organic phase was again evaporated and the residue dissolved in 1.5 ml of extensively dried MEK. The solution of ^{99m}Tc in MEK was transferred dropwise to a glasstube containing 100 mg of micronised, spheronised lactose monohydrate with a mass median diameter (MMD) of about 3 μm (AstraZeneca Liquid Production, Sweden), placed in an ultrasonic bath thermostated to $+30$ °C. The suspension was then dried under a stream of nitrogen until dryness (for about 30 min). The powder was labelled to a radioactivity concentration of about 9 MBq (^{99m}Tc)/mg of lactose at the time of administration to the first subject on a study day. It did not contain any detectable residues of solvents (MEK or tertiary butanol). An empty Turbuhaler inhaler was filled with about 75 mg of the ^{99m}Tc-labelled lactose.

The extent to which ^{99m}Tc delivered as ^{99m}Tc-labelled lactose would act as a marker for unlabelled lactose delivered from an identical dry powder inhaler was evaluated in vitro using a multi-stage liquid impinger (MLI) (Ph. Eur. 1997) at each of four target flows (30, 40, 60 and 80 l/min) selected for the in vivo study. The flow through the MLI was set with the inhaler inserted into the entrance port. The size bands for the standard flow (60 l/min) were: stage 1, >13 μm; stage 2, 6.8–13 μm; stage 3, 3.1–6.8 μm; and stage 4 (filter), <3.1 μm. Changes in flow (Q) altered the cut-off values by a factor $(Q/60)^{-1/2}$.

For each of the flows that were to be used in vivo, one inhaler with ^{99m}Tc-labelled lactose and one with unlabelled lactose were prepared and doses 21–30, 41–50 and 61–70 analysed (normally the inhaler contains 200 doses). The fine particle fraction (FPF), i.e. the fraction of dose representing particles smaller than 5 μm, was calculated using logarithmic interpolation between the stages. The distribution of radioactivity delivered as ^{99m}Tc-labelled lactose was analysed and compared with the distribution of unlabelled lactose from the same batch and delivered from an identical inhaler. The three impaction stages, the filter and the inhaler mouthpiece were washed with distilled water containing glucose as an internal standard. Samples of ^{99m}Tc-labelled lactose were analysed in duplicate for radioactivity using a gamma counter, and samples of ^{99m}Tc-labelled as well as unlabelled lactose were analysed in duplicate for lactose using liquid chromatography. In addition, mass median aerodynamic diameter (MMAD) of radioactivity was estimated.

One inhaler of ^{99m}Tc-labelled lactose was produced on each labelling occasion. Ten priming doses were sucked out at 60 l/min using a vacuum pump and discarded. The next 10 doses were used to measure the distribution of radioactivity using the MLI at 60 l/min as a quality control.

2.2. Subjects

Eight healthy men participated in this open, crossover and randomised study. Their mean age was 23 years (range: 19–29 years), body weight 74 kg (67–82 kg) and height 179 cm (173–186 cm). They were all non-smokers. Forced expiratory volume in 1 s was on average 94% (82–110%) and vital capacity 95% (80–120%) of predicted normal values (Berglund et al., 1963). The study was performed in accordance with the principles stated in the Declaration of Helsinki. Approvals were obtained from the Research Ethics Committee at the University of Lund/Malmö, Sweden, and the Committee for Radiation Protection at Malmö University Hospital, Sweden.

2.3. Adminstion of radioaerosol

Single doses of ^{99m}Tc-labelled lactose were given at weekly intervals. The subjects were studied in groups of up to four. They were given 100 mg of potassium iodide about 30 min prior to the inhalation of radioaerosol to block thyroid uptake of ^{99m}Tc-pertechnetate. They were then carefully trained in the inhalation technique using a placebo Turbuhaler inhaler. The radioaerosol was inhaled by subjects in an upright position. Inspiratory flow and volume were measured using a computerised Fleisch pneumotachograph flowhead (Vitalograph Ltd., UK). The subjects were instructed to take a full breath from residual volume to total lung capacity. Peak inspiratory flows (PIFs) of 30, 40, 60 and 80 l/min reached within 0.5 s after commencing inhalation were aimed at. The subjects were instructed to exhale gently through the nose after completed inhalation.

Each single dose of ^{99m}Tc-labelled lactose consisted of 10–15 consecutive inhalations (Table 1) and delivered a total amount of radioactivity of about 6 MBq of ^{99m}Tc to the lungs. After the last inhalation of ^{99m}Tc-labelled lactose, subjects were instructed to gargle the mouth thoroughly with 250 ml of water (collected for subsequent mea-

Table 1
Mean values (SD) for PIF and inhaled volume for inhalations of radioaerosol

	Target PIF (l/min)			
	30	40	60	80
PIF (l/min)	32.8 (0.8)	42.5 (0.4)	62.5 (2.4)	76.5 (7.1)
Inhaled volume (l)	3.9 (0.9)	3.9 (0.9)	3.8 (0.8)	3.2 (1.1)
n	15	11	11	10

n, median number of inhalations.

surements of radioactivity) and then to swallow another 250 ml of water. The inhaler mouthpiece was collected for subsequent measurements of radioactivity and a new mouthpiece attached to the inhaler before use by the next subject.

2.4. Scintigraphic measurements

Immediately following the inhalation of radioaerosol, posterior and anterior views of the thorax and upper abdomen, as well as posterior, anterior, and lateral views of the head were obtained with the subject in a sitting position. All emission images were acquired over 2 min using a one-head gamma camera (Maxi Camera 400, General Electric, Milwaukee, Wisconsin) fitted with a low-energy collimator. All images were stored in a 128×128 matrix. Lung image acquisition was completed within less than 5 min and stomach image acquisition was completed within 10 min after the last inhalation of radioaerosol. No spontaneous coughs were registered during any of the acquisition periods. Radioactivity in the mouth rinsing fluid and inhaler mouthpiece was determined using the same camera.

The contours of the lungs were assessed from a transmission scintigram obtained using a flood source of ^{57}Co placed over the anterior aspect of the subject's chest, with the gamma rays transmitted through the thorax and detected over 3 min by the gamma camera in the posterior view. Regions of interest within the lungs were delineated in the transmission scintigrams and projected onto the emission scintigrams to determine regional distribution of radioaerosol. Proper alignment of scintigrams was ensured by point sources of ^{57}Co placed on the subjects' shoulders. The transmission image was compared with an image of the flood source alone, so that the percentage transmission for each region could be derived and tissue attenuation of gamma rays in the lungs, oral cavity, larynx, and stomach corrected for. Background radiation was assessed in regions drawn over the shoulders, excluding point sources.

Radioactivity in each of the selected regions of interest in the body was calculated as the geometric mean of anterior and posterior counts, after subtraction of background radiation and correction for tissue attenuation. Radioactivity in the mouth rinsing fluid and inhaler mouthpiece was calculated on the basis of counts collected in one view. Only the right lung was analysed to evaluate pulmonary delivery patterns, as analysis of the left lung would present difficulties, due to interference from swallowed radioactivity in the stomach. Thus, pulmonary deposition was calculated as the percentage of radioactivity in the right lung multiplied by a factor of 1.9, based on the assumption that in healthy subjects, aerosol deposition is proportional to ventilation and ventilation to the right lung is equal to 52.5% of total ventilation (Arborelius et al., 1970). The deposition in each region was expressed as a percentage of the total measured radioactivity in the body, mouth rinsing fluid and inhaler mouthpiece. Regional distribution of radioactivity within the airways was established both as a PI (i.e. the ratio of peripheral to central lung zone deposition) and as count density distribution (the coefficient of variation of counts per pixel) in the right lung. Lung zones were defined in concordance with Olséni et al. (1994).

2.5. Statistical tests

An analysis of variance model was used to investigate the effect of PIF on total pulmonary deposition, peripheral pulmonary deposition, central pulmonary deposition, PI, and coefficient of variation in counts per pixel in the whole lung, all estimated on the basis of right lung data. A P-value of < 0.05 was considered to indicate statistical significance.

3. Results

The size distribution of radioactivity delivered as ^{99m}Tc-labelled lactose at each of the four in vivo target flows is shown in Fig. 1. MMAD of radioactivity was estimated at about 3.3, 2.7, 2.3 and 2.2 μm for 30, 40, 60 and 80 l/min, respectively. The ratio of the FPF of radioactivity to the FPF of unlabelled lactose was 2.1, 1.6, 1.2 and 1.5 for 30, 40, 60 and 80 l/min, respectively (Fig. 2). The relative standard deviation of the amount of

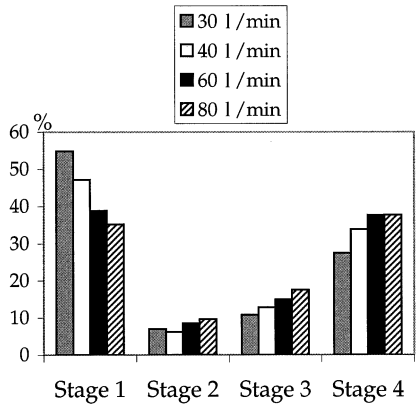

Fig. 1. Distribution of radioactivity delivered as ^{99m}Tc-labelled lactose at four different flows through the MLI (% of delivered dose on the four MLI stages).

radioactivity detected on each stage of the MLI was 16.7% (stage 1), 12.9% (stage 2), 9.8% (stage 3), and 10.9% (filter). The variation in FPF of radioactivity delivered as ^{99m}Tc-labelled lactose at 60 l/min was 12% between study inhalers ($n = 13$) calculated as the relative standard deviation of the mean.

The radioaerosol was inhaled well in accordance with instructions. For target PIFs of 30, 40 and 60 l/min, achieved PIFs were marginally higher than the targets, whereas for the target PIF of 80 l/min, achieved PIF was marginally lower

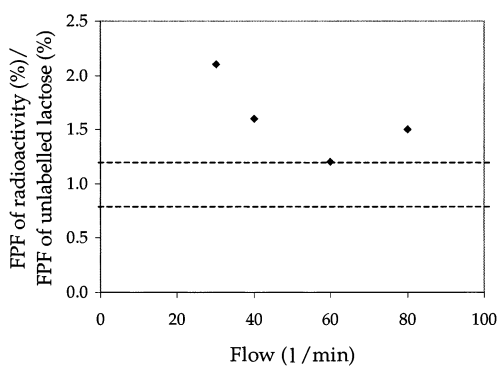

Fig. 2. Ratio of the FPF of radioactivity (% of dose delivered as ^{99m}Tc-labelled lactose) to the FPF of lactose (% of dose delivered as unlabelled aerosol) at four different flows through the MLI. Horizontal lines indicate acceptance limits of 0.8 and 1.2.

(Table 1). Mean inhaled volume ranged from 3.2 to 3.9 l.

The total pulmonary deposition of radioactivity was estimated at 17% of the total measured radioactivity in body regions, mouth rinsing fluid, and inhaler mouthpiece after inhalation at a target PIF of 60 l/min. A change in PIF to 30, 40 or 80 l/min did not change total pulmonary deposition to any statistically significant extent. The largest fraction, 56–65%, was deposited orally, i.e. found in the stomach, oral cavity and mouth rinsing fluid. Only 1% was deposited in the larynx. Between 10 and 20% was retained in the inhaler mouthpiece. Visual inspection of lateral head images indicated that the oral cavity was lined by radioactivity to about the same extent, irrespective of PIF.

Both the PI and the count density distribution seemed to remain unchanged with varying PIF (Fig. 3). No statistically significant differences were found, either in PI or in count density distribution.

4. Discussion

This study demonstrated the potential risk of producing a radiolabelled dry powder aerosol with aerodynamic properties different from those of the original, unlabelled one. However, discrepancies may not be detectable when using standard conditions for in vitro testing. Thus, whenever pulmonary deposition of an inhaled drug product is to be estimated in vivo by the use of a radiolabelled version of that product, it appears essential to perform adequate in vitro validation of the radiolabelled formulation prior to initiating the in vivo study. All conditions relevant to the in vivo situation should be examined to adequately measure the in vitro characteristics of a dry powder radioaerosol and the corresponding unlabelled aerosol. This underlines the importance of the recently published standards for validation of radiolabelling methods for pharmaceutical aerosols (Snell and Ganderton, 1999). The size distribution of radioactivity delivered as a ^{99m}Tc-labelled lactose dry powder was close to that of lactose delivered as the corresponding unlabelled

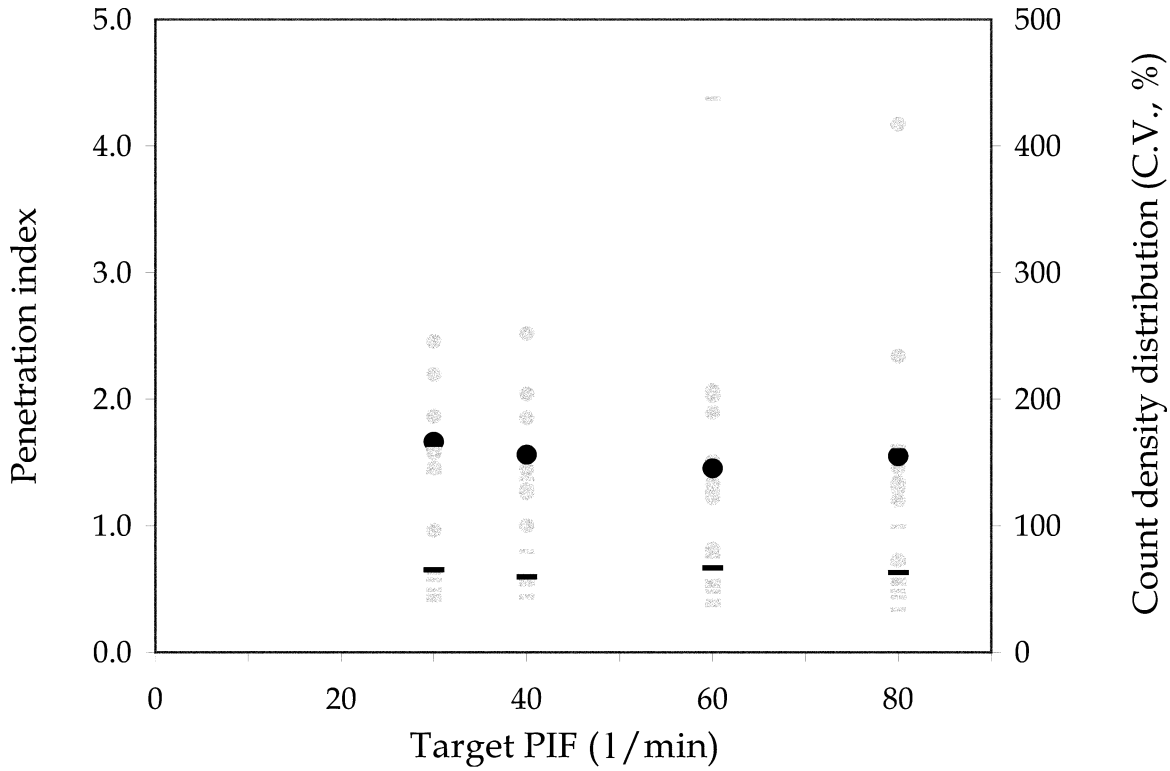

Fig. 3. Individual data points and geometric means for PI (●) and count density distribution (▬), expressed as the coefficient of variation, CV (%), of counts in the lung.

aerosol when analysed at a moderate in vivo target flow, 60 l/min, but mismatches where found when using any of the slower (30 and 40 l/min) or faster (80 l/min) in vivo target flows. For each of the latter flows, the ratio of the FPF of radioactivity to the FPF of unlabelled lactose exceeded the upper limit of the acceptance range, 0.8–1.2, used for radiolabelling methods (Snell and Ganderton, 1999). Thus, the radionuclide could not be considered as a valid marker of unlabelled lactose. However, as the primary objective of this study was to investigate whether or not the intrapulmonary distribution of aerosol is affected by the inspiratory effort exerted by the subject while inhaling a dry powder radioaerosol, the radiolabelling method was still considered valid for this end-point to be investigated. The radiolabelling method was considered reproducible on the basis of the fairly small variation (12%) in FPF of radioactivity.

Two indices of regional distribution were assessed in this study: PI and count density distribution. They have previously been shown to correlate quite well (Olséni et al., 1994). In this study, both indices were found to be consistent over the range of PIFs. This finding is in agreement with that of a previous study in which ^{99m}Tc-labelled budesonide inhaled via Turbuhaler at two PIFs, 35 and 60 l/min, resulted in PIs of about 1.8 and 1.7 (Borgström et al., 1994). A PI of about 1.7 has also been reported for two nebulised aerosols: ^{99m}Tc-labelled albumin microspheres (MMAD 5 μm) and ^{99m}Tc-DTPA (MMD 2 μm) after administration to healthy, non-smoking subjects (Olséni et al., 1994; Wollmer and Evander, 1994). As the scintigraphic method is sufficiently sensitive to allow differences in regional distribution of radioaerosol to be detected between patients with obstructed airways and subjects with normal lung function (Dolovich et

al., 1976; Olséni et al., 1994), it is likely that variations in particle size distribution within the range of 2–5 μm do not cause any major changes in regional distribution of radioaerosol in healthy airways. In this study, in which the MMAD of radioactivity delivered as ^{99m}Tc-labelled lactose varied between 3.3 and 2.2 μm (as indicated by the size distribution measurements), the PI was found to be about 1.6, irrespective of PIF. This indicates that the regional distribution of aerosol in healthy airways may be insensitive to large differences in inspiratory flow during the inhalation of aerosol. However, it should be noted that the decrease in median particle size observed with increasing flow would tend to offset any increased propensity for radioaerosol to impact in the central lung as a result of the increasing flow. To further investigate the hypothesis that regional distribution is little affected by inspiratory flow rate, investigations involving a number of radioaerosols with different flow-independent particle size distributions would be needed. Our measurements showed that the regional distribution of radioactivity within the airways was independent of inspiratory effort for the tested radioaerosol. The possibility to extrapolate the finding to other formulations is limited by the poor agreement between the size distribution of radionuclide and unlabelled lactose at flows other than 60 l/min and the fact that other formulations cannot be presumed to exhibit a similar effect of flow on median particle size.

In order to adequately quantify intrapulmonary distribution of radioaerosol, the amount of radioactivity reaching the lungs had to be as large as possible, while still safe to inhale. It was estimated that pulmonary delivery per subject and study day was around 6 MBq of ^{99m}Tc, which is of the same order as that usually recommended for planar imaging studies (Chan, 1993; Newman, 1993). This resulted in a measurement error of less than 2% for any individual peripheral or central lung zone. A great number of consecutive inhalations had to be administered. This did not raise any concerns regarding safety as lactose is widely used as an excipient in aerosol formulations and has proved harmless, even in large amounts (Shaw et al., 1994). However, pulmonary clearance mechanisms account for rapid removal of ^{99m}Tc (as pertechnetate) from the lungs with a half-life of about 10–11 min (Monaghan et al., 1991; Fanti et al., 1996). Thus, it is likely that a portion of the radioactivity delivered to the lungs by the first inhalations was distributed to sites outside the regions of interest, and therefore not captured in the scintigram.

In conclusion, this study demonstrated the importance of using conditions relevant to the in vivo situation in the in vitro measurements to adequately validate dry powder radioaerosols. Two regional distribution indices measured in vivo indicated that regional distribution of aerosol in healthy airways was insensitive to differences in the inspiratory effort exerted by the subject while inhaling the experimental dry powder radioaerosol.

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