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An alternative to direct labelling of pressurised bronchodilator aerosol

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Summary

A method of mixing radiolabelled teflon particles with micronised salbutamol in a reconstituted pressurized cannister for drug deposition pattern and lung functions studies is described. A method of quality controlling the reconstituted cannister is also described. Deposition pattern studies performed on 6 subjects (1 normal, 5 asthmatics) showed that 12.7% of the drug was deposited in the lungs, 76.5% in the throat and stomach and 10.8% in the actuator. FEV_1 improved by 25.4% in 5 asthmatics. This technique serves as an alternative to direct labelling of any pressurised drug particles for deposition pattern and lung function studies especially when a direct labelling technique is not readily available.

Introduction

Recent developments in aerosol radiolabelling techniques have provided an understanding of the deposition pattern of various inhaled substances in the respiratory tract. To study a particular aerosolised bronchodilator drug, ideally the drug should be directly labelled so that both deposition patterns and bronchodilator responses can be assessed at the same time. Unfortunately, a direct labelling technique is either not readily available or is complicated, such as that for ipratropium bromide (Short et al., 1981). Because of the difficulty in direct labelling, technetium-99m labelled teflon particles with size distribution similar to that of a bronchodilator drug were used in a reconstituted cannister in place of the bronchodilator drug for deposition pattern studies (Newman et al., 1981). This technique has the disadvantage in that the bronchodilator response cannot be studied at the same time. To overcome this problem, we have developed a technique which involves the mixing of technetium-labelled teflon particles with a bronchodilator drug in a reconstituted cannister so that both deposition pattern and bronchodilator responses can be assessed together. An outline of the technique, its quality control and the results of studies on 6 subjects are described in this paper.

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Materials and Methods

Reconstitution of pressurised cannister

Technetium-99m labelled teflon (fluorinated ethylene propylene) particles were produced using the method which has been previously described (Camner and Philipson, 1971; Newman et al., 1981) which some modifications. A spinning-disc aerosol generator was set up in a leaded-glass tank. Technetium-99m pertechnetate in 10 ml normal saline was passed through a cation exchange column (Amberlite 120) and the eluate collected into a glass container. This was evaporated to dryness by heating in a hot water bath and blowing with air at the same time. These processes remove both NaCl and HCl which might cause leaching of technetium. 0.5 ml of a submicron teflon suspension in 50 ml of 40% ethanol was added to the technetium residue and the solution run onto a spinning disc revolving at 62,000 rpm.

A 240 W light serving as a heat source was placed in the tank to enhance the evaporation of liquid-droplets. The particles generated were collected by scraping glass collection plates on the base of the tank and placing them into a cannister. This was subsequently heated to 240 °C to improve the durability of the particles. The cannister was then cooled to −60 °C by immersion in solid CO_2. A commercial cannister containing micronised salbutamol, chlorofluorocarbon propellants and surfactant was cooled down at the same time to convert the propellants into liquid form. Next, the top of the commercial cannister was quickly removed by a special blade and the content transferred into the cannister containing the teflon particles. A valve mechanism was quickly secured in position and the cannister crimped. The process of transferring the contents and securing the valve was completed in less than 10 s to prevent the evaporation of propellants. The reconstituted cannister was then agitated in an ultrasonic bath for 10 min to disperse the particles.

Quality control analysis

Aluminium foil placed on the base of the tank was used to collect samples of settling teflon particles for size analysis by electron microscopy. Salbutamol particles were analysed separately by the manufacturer using a Malvern Particle Analyser.

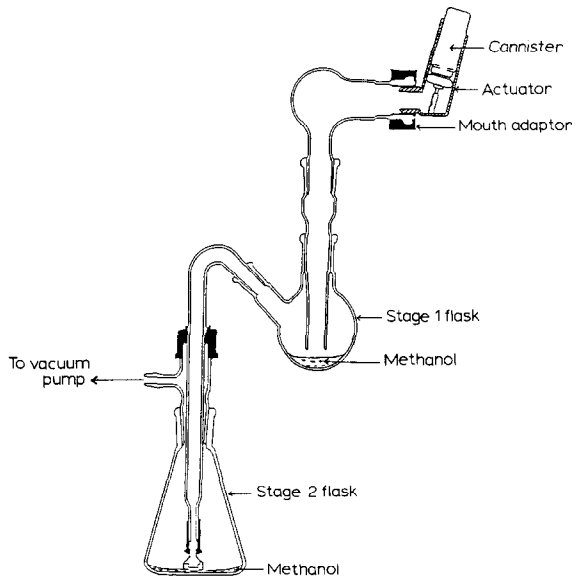

Fig. 1. Schematic representation of a twin impinger (Glaxo) with a metered-dose inhaler in position.

The reconstituted cannister was examined by firing 10 puffs into a twin impinger (Glaxo). The two-chamber instrument had a 50% effective cut-off diameter (ECD) of 6.4 μm so that particles of this aerodynamic size have a 50% probability of penetrating to the lower chamber. Particles of smaller size have a higher probability of penetration and those larger than 6.4 μm have a smaller probability. 7 and 30 ml of methanol were placed in stage 1 and 2 respectively for collection of particles (Fig. 1). The impinger was operated by a vacuum pump operating at 60 litres/min. After actuation, the particles deposited on the actuator and valve and at stages 1 and 2 were assessed for radioactivity using a gamma-camera and by assaying for salbutamol spectrophotometrically after the radioactivity had completely decayed.

Deposition pattern studies

For deposition pattern studies in 6 informed subjects, 10 GBq of technetium-99m was initially used during the teflon particle production. After collection by scraping, about 200–400 MBq of labelled teflon particles were available for use.

TABLE 1

Mean ± S.D. percentage deposition of salbutamol and radioactivity in actuator, stage 1 and 2 of twin impinger before and after heating the tank

	Pre-heating			Post-heating		
	Actuator	Stage 1	Stage 2	Actuator	Stage 1	Stage 2
Salbutamol	20.7 ± 6.0	38.7 ± 6.3	40.7 ± 4.1	9.7 ± 2.5	45.0 ± 4.3	44.7 ± 4.7
Radioactivity	19.7 ± 5.9	59.1 ± 6.3	21.1 ± 3.0	10.2 ± 3.0	52.7 ± 6.0	37.1 ± 6.8

These were mixed with salbutamol in an 80-dose cannister. Each actuation contained 100 μg of salbutamol and 2–4 MBq of radioactivity. Four puffs were given to each of the 6 subjects (1 normal, 5 asthmatics) after careful instruction and supervision regarding the technique of inhalation. The metered dose inhaler was actuated at the beginning of a slow but deep inhalation followed by a 10-s breath-hold. There was a 30-s lapse in between actuations.

Immediately after the inhalation, subjects were imaged seated in front of a dual-headed gamma-camera which acquired simultaneous anterior and posterior views. Images of the lungs and stomach were collected for a count limit of 400 K and those of the throat (if not included in the lung view) for a time limit of 120 s. Radioactivity retained in the valve and actuator was collected by cleaning them with methanol and the solution counted for a time limit of 100-s. The camera was

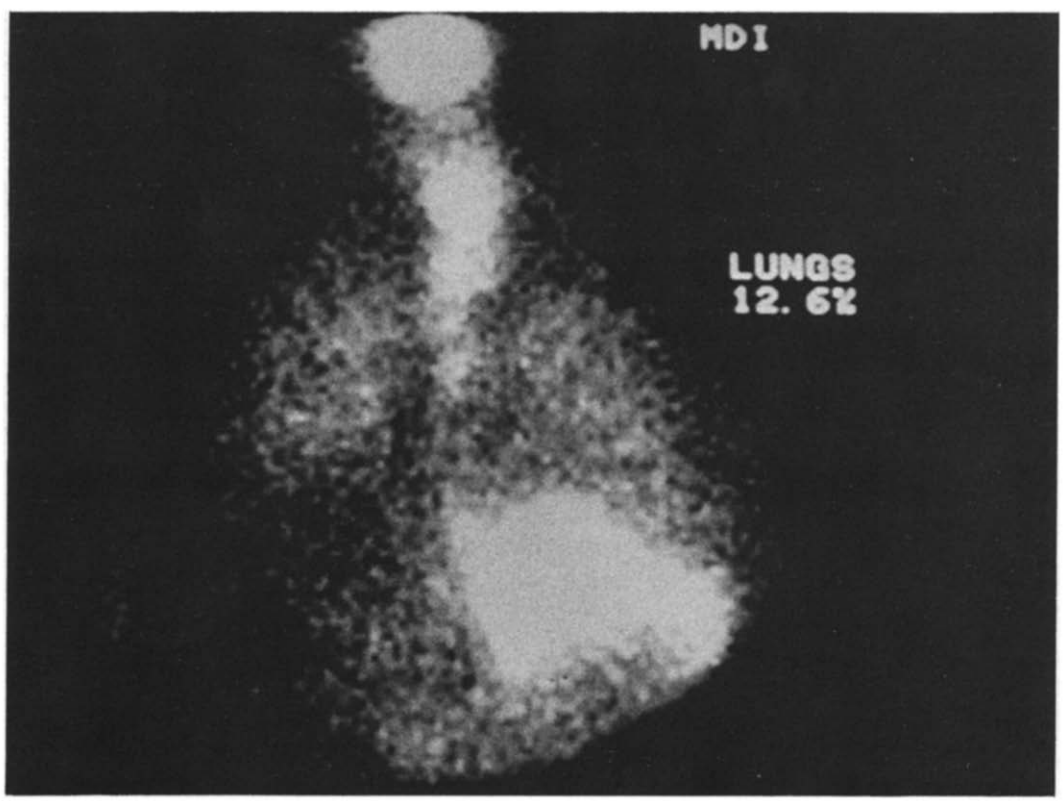

Fig. 2. A gamma-camera picture of one of the subjects showing deposition pattern in the throat, lungs and stomach.

interfaced with an ADAC computer and counts in the regions of interest (ROI) delineating the lungs, mediastinum, throat and stomach were obtained. The values were expressed as percentages of total deposition in the body, valve and actuator after correcting for background activity, attenuation of photons in chest wall, neck and abdomen and also radioactive decay. Spirometry was also performed on asthmatic subjects before and 30 min after inhalation of aerosol.

Maximum absorbed radiation dose to each subject was about 1 mGy to the lungs and 0.05 mGy to the whole body. The study was approved by the Ethics Committee of University College and Middlesex School of Medicine.

Results

Particle sizes of teflon after settling and scraping were similar. 100% of teflon mass was attributable to particles of 4.0 μm diameter or less as compared to 99.4% of salbutamol mass. The mass median diameter (MMD) of teflon was 2.1 μm (S.D. $\pm$ 1.5 μm) and that of salbutamol was 1.45 μm (S.D. $\pm$ 1.4 μm).

The percentage depositions of teflon, as measured by radioactivity, and salbutamol, as assayed spectrophotometrically, in the actuator, stages 1 and 2 of twin impinger, are shown in Table 1. Prior to heating the tank, the teflon particles collected were wet and this resulted in a lower deposition in stage 2 (21.1 $\pm$ 3.0%); this improved (to 37.1 $\pm$ 6.8%) after heating. The percentage of salbutamol in stage 2 was 44.7 $\pm$ 4.7%. The difference between radioactivity and salbutamol was small and considered acceptable. The mass of drug released per actuation ranged from 96.8 μg to 105.4 μg (mean $\pm$ S.D., 101.6 $\pm$ 2.9 μg) compared with 100 μg as specified by the manufacturer.

Table 2 shows the results of the deposition pattern studies in the 6 subjects and the improvement in FEV_1 in the 5 asthmatics following the inhalation of 400 μg salbutamol mixed with technetium-99m labelled teflon. Fig. 2 shows a gamma-camera picture of one subject illustrating the typical deposition pattern in the throat, lungs and stomach.

Discussion

Our technique involves the mixing of technetium-99m labelled teflon and salbutamol particles in a reconstituted pressurised cannister containing chlorofluorocarbon propellants and surfactant. The advantage of this technique is that it permits both the deposition pattern study and the measurement of bronchodilator response to be performed at the same time. For a reliable result, it is important not only to match the particle size of teflon as closely as possible to that of salbutamol, but also to make sure that the two types of

TABLE 2

Percentage deposition of aerosol at various sites and changes in FEV_1 following the inhalation of pressurised aerosol in 6 subjects

M = male; F = female.

Subject, age, sex	Actuator (%)	Lung (%)	Throat + stomach (%)	Baseline FEV_1 (l)	Max. FEV_1'	% FEV_1
(1) * 30 M	8.2	12.8	79.0	–	–	–
(2) 20 M	12.5	16.0	71.5	2.0	2.65	32.5
(3) 66 F	15.7	12.3	72.0	1.15	1.40	21.7
(4) 53 M	14.7	10.2	75.1	1.70	1.85	11.5
(5) 58 F	7.9	12.5	79.6	1.50	1.75	16.7
(6) 61 F	5.7	12.2	82.1	1.10	1.55	40.8
Mean	10.8	12.7	76.5	1.49	1.84	24.7
(S.D.)	(4.1)	(1.9)	(4.3)	(0.38)	(0.48)	(11.9)

* Normal subject.

particles have similar aerodynamic behaviour. We have shown that, although the particle sizes are not 100% matched, probably partly because of the different sizing techniques used, they behave aerodynamically similarly when assessed on the twin impinger which can be considered to serve as a model of the respiratory tract. It is therefore highly likely that the teflon and salbutamol deposit similarly in vivo.

Apart from using a high rotor speed in order to produce small enough teflon particles for our purposes, a heat source in the tank was employed while spinning to facilitate the evaporation of liquid drops. Incomplete evaporation was thought to cause agglomeration of the particles and hence result in a lower deposition in stage 2 of the twin impinger. This has also been previously suggested (Hurford, 1981). The percentage deposition in stage 2 approaches that of salbutamol when heating was employed during spinning. Using our technique, 12.7% of the actuated dose deposits within the lungs, 10.8% in the actuator and 76.5% in the throat and stomach. Our results agree with previous indirect estimates (Davies, 1975), deposition pattern studies using radioisotope (Newman et al., 1982; Spiro et al., 1984; Vindgren et al., 1987), although the percentage of lung deposition is smaller than the result of one other study (Kohler et al., 1988).

We feel that this technique can be applied to any pressurised aerosol drug especially when a direct labelling technique is not readily available.

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