BRIEF COMMUNICATIONS

Characterization of the Generation of Radiolabeled Monodisperse Albuterol Particles Using the Spinning-Top Aerosol Generator

Omar S. Usmani, MB BS[1]; Martyn F. Biddiscombe, PhD[2]; S. Richard Underwood, MD[2]; and Peter J. Barnes, MD[1]

[1]Department of Thoracic Medicine, National Heart and Lung Institute, Imperial College London, London, United Kingdom; and
[2]Department of Nuclear Medicine, Royal Brompton Hospital, London, United Kingdom

Inhaled radiolabeled aerosols provide invaluable information about in vivo drug deposition. Here, we report our methodology for radiolabeling and imaging monodisperse pharmacologic aerosols in order to study basic aerosol science concepts of drug delivery within the human airways. **Methods:** We used a spinning-top aerosol generator to produce ^{99m}Tc-labeled monodisperse albuterol sulfate aerosols of 1.5-, 3-, and 6-μm mass median aerodynamic diameter. **Results:** In vitro Andersen cascade validation data showed that technetium and albuterol were coassociated on each impactor stage for all 3 aerosols, and the radiolabeling process itself did not affect their particle size distributions. Good-quality γ-camera scintigraphic images of lung and extrathoracic deposition were obtained within an asthmatic patient. **Conclusion:** We have successfully radiolabeled and imaged monodisperse albuterol aerosols within the human lungs. This novel technique provides an important tool to relate fundamental concepts of aerosol particle behavior, in vivo deposition, and therapeutic clinical response.

Key Words: lung aerosol deposition; β_2-agonist; particle size; planar scintigraphy

Radiolabeling methods have contributed valuable information about the deposition characteristics of inhaled drugs within the human lung and provide a quantitative assessment of the site and extent of in vivo distributions that is difficult to obtain by other means (*1–3*). Therapeutic agents used in the treatment of airway disease have been radiolabeled in metered dose inhalers and dry powder inhalers, by which important information about device delivery characteristics and drug formulations has been obtained (*4–6*). Polydisperse aerosols, however, because of their wide size distributions, cannot address fundamental questions on particle behavior within the lungs (*7*). In contrast, monodisperse aerosols are ideal for studying the effects of drug particle size within the airways, as their narrow size dispersity (geometric SD [GSD] < 1.22) allows the investigation of individual particle sizes. Although inert radiolabeled monodisperse compounds have been widely used in inhalation aerosol research (*8–10*), no studies to date have radiolabeled therapeutic monodisperse aerosols and observed their deposition behavior within the human lungs. Such a potentially valuable tool may allow us to explore important in vitro basic aerosol science concepts of drug delivery within the human airways in vivo.

The aim of this research was to demonstrate an effective and versatile method for radiolabeling stable, pharmacologically active, monodisperse drug particles of various sizes and to acquire good-quality scintigraphic images of lung and extrathoracic deposition. To generate radiolabeled particles of 1.5-, 3.0-, and 6.0-μm mass median aerodynamic diameter (MMAD), we adapted our previously described methods for producing unlabeled monodisperse albuterol sulfate aerosols using a spinning-top aerosol generator (STAG) (*11*).

MATERIALS AND METHODS

We adhered to a recent consensus statement on standards for the experimental validation of the method of radiolabeling therapeutic compounds and their use in scintigraphic studies (*12*).

Preparation of Technetium-Labeled Monodisperse Albuterol Aerosols

We have adapted our system for generating and delivering unlabeled monodisperse albuterol, which has been described in detail elsewhere (Fig. 1) (*11*). Briefly, the system consists of the STAG (Mark II; Research Engineers Ltd.) with an air-driven spinning disk, a chamber for accumulation of the generated aerosol, and an inhalation control unit for regulating aerosol delivery to the patient. A syringe driver delivers drug solution to the center of a spinning disk, and by altering the disk speed, one can generate various-sized particles. Primary droplet diameter is inversely proportional to disk rotational speed, and unwanted secondary droplets are removed by the inbuilt exhaust system. The MMAD, GSD, and drug concentration of the generated aerosol particles are
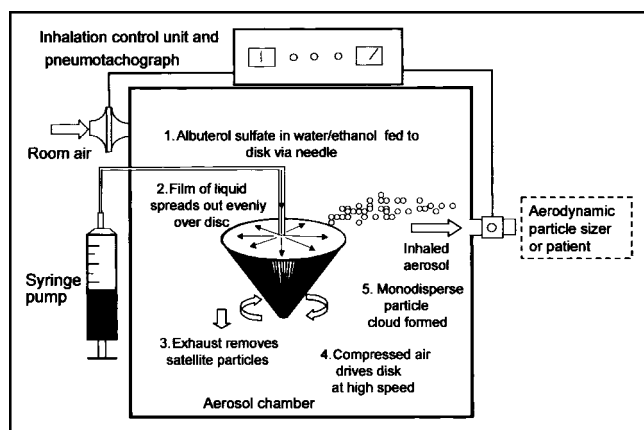
FIGURE 1. Simplified schematic diagram showing principle of operation of aerosol generation and delivery system, incorporating STAG disk, inhalation control unit, inhalation chamber, and position of aerodynamic particle sizer or patient.

characterized using an aerodynamic particle sizer (model 3302; TSI Inc.) attached to the mouthpiece of the aerosol chamber.

Table 1 specifies the materials, quantities, and STAG disk rotor speeds used to generate the 3 radiolabeled particle sizes: 1.5, 3, and 6 μm. Albuterol sulfate powder (GlaxoSmithKline) was weighed into a lead-shielded scintillation vial, into which a measured quantity of technetium pertechnetate ($^{99m}TcO_4^-$) in normal saline, eluted from a molybdenum/technetium generator (Mallinckrodt, Radiopharmacy Services, Middlesex Hospital), was added. For in vitro validation experiments, 300 MBq of radioactivity was used, and for in vivo imaging, this was increased to 5 GBq. The solution was made up to a 1-mL volume with sterile water, when necessary, and the drug was allowed to completely dissolve before ethanol (96% pharmaceutical grade, Martindale brand) was added. The resulting solution was drawn into a 50-mL syringe and aerosolized in the STAG to generate dry and physically stable monodisperse albuterol aerosols (11). Lead shielding was used at all stages.

Andersen Cascade Impactor In Vitro Validation

The purpose of this experiment was to ensure that the radionuclide ^{99m}Tc acted as a valid marker for the albuterol particles and that both drug and radiolabel were coassociated. Radiolabeled aerosols were drawn through an Andersen cascade impactor (Graseby-Andersen) connected to the mouthpiece of the STAG chamber for 3 min at a flow rate of 28.3 L/min using a pump (Platon). Each impactor stage was washed, using a methanol:water solution (70:30), into separate glass volumetric flasks and made up to a final volume of 50 mL. Radioactive counts in each flask were obtained by counting for 60 s under a γ-camera (Sopha Medical). After the radioactivity had been allowed to decay for at least 48 h, the mass concentration of albuterol in each flask was measured using an ultraviolet spectrophotometer (Kontron Instruments). The amounts of drug and radioactivity on each stage were expressed as a percentage of their respective totals recovered from all the impactor stages.

We undertook 3 runs for each aerosol size. The first 2 runs used 300 MBq of radioactivity so as to minimize operator-handling exposure. For the third run we used approximately 5 GBq of radioactivity decayed by 24 h, which was representative of the in vivo clinical imaging study-dose. In addition, unlabeled drug distributions with MMADs of 1.5, 3, and 6 μm were separately generated and sampled by the Andersen cascade impactor and were compared with size distributions of the labeled drug and radioisotope (Fig. 2).

Imaging an In Vivo Distribution of Radiolabeled Monodisperse Albuterol

To evaluate the effectiveness of the radiolabeling method, a clinically stable asthmatic woman (aged 35 y; forced expiratory volume in 1 s, 60% of that predicted) took part in a preliminary study. The study was approved by the Ethics Committee of the Royal Brompton and Harefield Hospital National Health Service Trust, and the patient gave written informed consent. Our objective was to deliver an exact drug mass to the patient with sufficient labeled radioactivity to achieve an adequate clinical image for subsequent analysis, without exposing the patient or the operator to a larger than necessary radiation dose. For planar imaging, as little as 1 MBq within the lungs is sufficient for generating a satisfactory lung image (13), although larger amounts will produce better count statistics. We delivered a 30-μg dose of albuterol sulfate in 3 sequential 1-L breaths (10 μg/L breath), with an MMAD of 3 μm and a GSD of 1.18, verified by the aerodynamic particle sizer. Each 1-L breath was inhaled from functional residual capacity in a controlled and identical manner at a flow rate of between 30 and 60 L/min as guided by a visual indicator, followed by a 10-s breath-hold pause. We calculated that a mass of 30 μg of albuterol aerosol particles would contain 3 MBq of ^{99m}Tc, if the initial radioactivity were 5 GBq in a starting drug mass of 50 mg (Table 1).

All imaging was undertaken in the Nuclear Medicine Department of the Royal Brompton Hospital. A single-head γ-camera (Sopha Medical) was used to acquire and process the radioaerosol distribution. The data were downloaded for further processing to a HERMES workstation (Hermes). Immediately after inhalation, patients were seated upright with their back against a large-field-of-view γ-camera equipped with a high-sensitivity parallel-hole collimator, with their lungs in the center of the field of view. Posterior thorax, anterior thorax, and lateral throat images were obtained as 1-min acquisition time frames over 4, 4, and 2 min, respectively, with the patients repositioned between images and asked to remain still to minimize movement artifacts. Imaging data were collected in 1-min time frames, so that any dissociation of the radiolabel could be observed. Images were recorded on a 64 × 64 matrix using the software of the camera and, after an acquisition time of 4 min, typically contained 80,000 counts.

TABLE 1
Materials and Quantities for Generation of the 3 Radiolabeled-Albuterol Particle Sizes

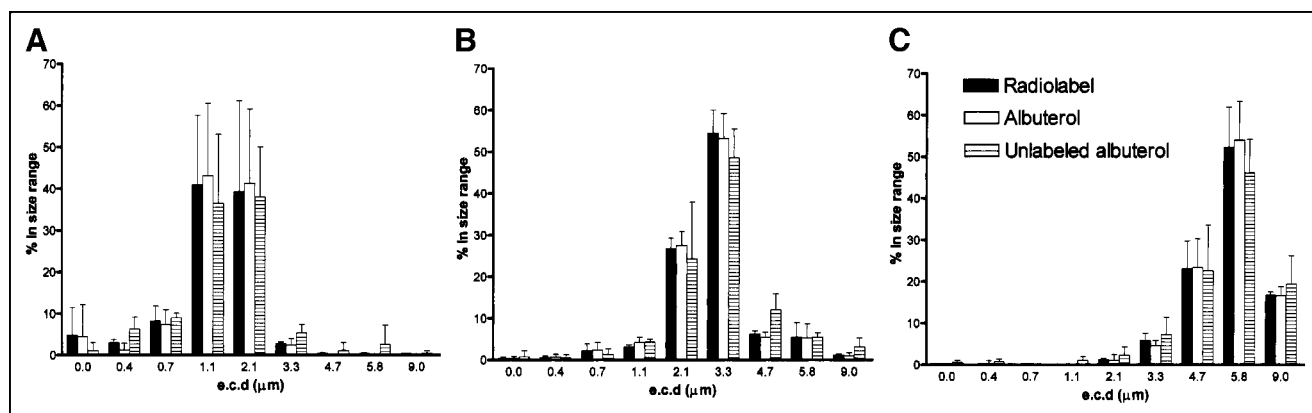| Materials and quantities	Monodisperse particle size (μm)		
	1.5	3	6
Albuterol sulfate (mg)	25	50	50
Sterile water (mL)	Up to 1	Up to 1	Up to 1
Ethanol (mL)	12.5	12.5	12.5
$^{99m}TcO_4^-$ (in vitro) ≤ 1-mL volume (MBq)	300	300	300
$^{99m}TcO_4^-$ (in vivo) ≤ 1-mL volume (GBq)		5	
STAG disk rotor speed	2,000	1,200	600

FIGURE 2. Mean percentage deposition of radiolabel, labeled albuterol, and unlabeled albuterol for aerosols with MMADs of 1.5 μm (A), 3 μm (B), and 6 μm (C), collected on the 9 stages of an Andersen cascade impactor operated at flow rate of 28.3 L/min. Effective cutoff diameters (e.c.d.) for the 8 impactor stages and backup filter are shown. Data are of 3 experiments, and error bars indicate SD of each value.

RESULTS

The Andersen validation experimental data showed that the levels of drug and radioactivity were closely associated and well matched on each impactor stage for the 3 monodisperse particle-size distributions (Fig. 2). They indicated that the albuterol sulfate particles carried the ^{99m}Tc-radionuclide into the Andersen cascade impactor, with both depositing on the 9 stages in similar proportions. Figure 2 illustrates that the distributions of radiolabel and drug were close to that of unlabeled albuterol sulfate, demonstrating that the radiolabeling process itself did not affect the particle-size distribution of the monodisperse aerosol cloud generated. We found no difference in the coassociation distributions whether 5 GBq decayed by 24 h or 300 MBq of radioactivity were used, implying that the amount of technetium had a negligible effect on the aerosol particle distributions. γ-Camera deposition images of the posterior and anterior thorax and lateral throat, obtained during a 10-min period from an asthmatic patient who inhaled a 3-μm ^{99m}Tc-labeled monodisperse albuterol aerosol, are shown in Figure 3.

The clearance data of ^{99m}Tc from the lungs, based on the 1-min time frames acquired for the posterior and anterior thorax images in the asthmatic patient, are represented in Figure 4. Because the radioisotope and albuterol have a simple physical association rather than a chemical bond, the two become separated after deposition within the lung, and the ^{99m}Tc becomes rapidly absorbed into the systemic circulation (14,15). The lung counts in each successive 1-min time frame progressively decreased after aerosol inhalation, and after 4 min approximately 20% of radioactivity had cleared from the lung field.

DISCUSSION

The successful radiolabeling of drug particles for inhalation depends on the ability to incorporate the radionuclide within the drug formulation and for it to remain with the drug until deposition within the airways (12). We have shown that ^{99m}Tc acts as a suitable marker for albuterol and matches the drug distribution in vitro for 3 monodisperse particle distributions generated using a STAG. The radiolabeling technique involves a physical association between
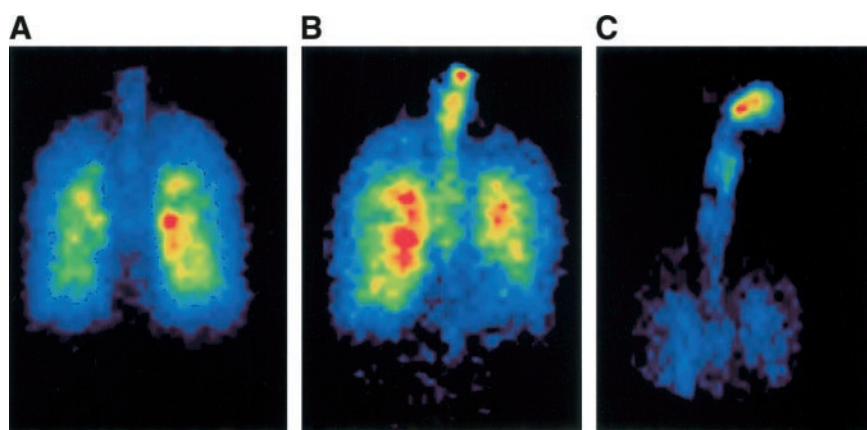

FIGURE 3. Posterior thorax (A), anterior thorax (B), and lateral throat (C) γ-camera images of asthmatic patient using ^{99m}Tc-labeled aerosol particles with MMAD of 3 μm (GSD, 1.18). Red areas indicate regions of highest radioactivity, and black, of least.
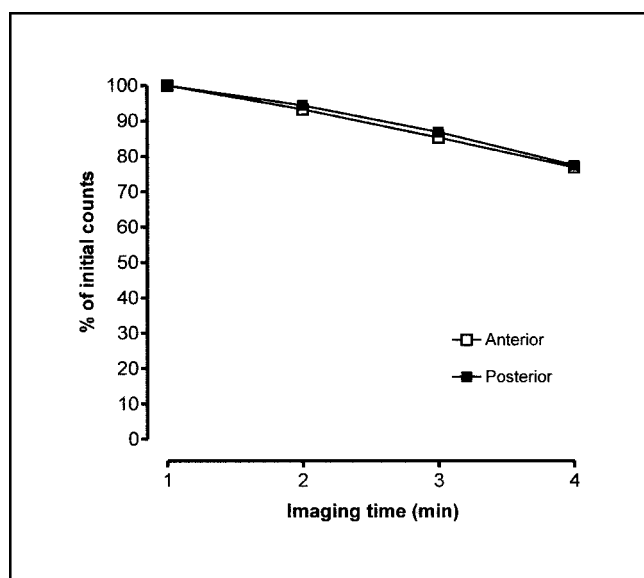
FIGURE 4. Clearance of ^{99m}Tc from lungs of asthmatic patient. Data at each 1-min image are shown as percentage of initial radioactivity counts for anterior and posterior images. This was calculated by dividing total acquired lung counts in each image by those obtained from first 1-min image.

drug and radiolabel (*16*). The principle is that the radionuclide and drug are homogeneously mixed in solution before delivery and remain so in each aerosol droplet formed. Subsequent evaporation of ethanol leaves airborne solid-drug particles with radionuclide distributed throughout. By comparing labeled drug and unlabeled drug cascade impactor distributions, we have demonstrated that the radiolabeling process itself does not affect the size distribution of the aerosol cloud generated. The small differences observed between the radiolabeled and unlabeled drug distributions resulted from the small variability in particle sizes generated by the STAG when the runs were repeated (*11*).

With our delivery system, an initial 5 GBq of radioactivity delivered a dose of 3 MBq in 30 μg of drug. It was not possible to use less radioactivity, because of the intrinsic losses within our delivery system. The aerosol chamber (150 L) acts as a holding reservoir, but aerosol may be lost on the walls or, more significantly, to the chamber base because of particle sedimentation under gravity. Aerosol particles greater than 1 μm, especially those of 6 μm, sediment relatively quickly once they are formed (*11*). To achieve the required dose, it is necessary to generate particles more quickly than they are being lost. Inevitably, more are generated than are delivered because of this effect. In addition, approximately 6% of the aerosol mass is generated in unwanted secondary "satellite" particles, which are removed through the exhaust system.

Disassociation of the ^{99m}Tc from the albuterol is an important consideration, as it limits the imaging time that may be used. Satisfactory images of initial airway deposition sites could be acquired, although we were unable to follow the biodistribution of albuterol in vivo to gain information on its lung redistribution, clearance, and uptake into the systemic circulation. However, even with these limits, invaluable information for assessing lung deposition may be obtained. The scintigraphic images obtained need to be corrected for tissue attenuation, background counts, radiation decay, and the depth of the source within the chest (*12*) before further analysis, in order to quantify airway deposition patterns of the drug–radionuclide combination. In addition, a clearance correction factor to allow for the rapid absorption of the radionuclide from the lungs into the bloodstream would need to be calculated, particularly for the second (anterior thorax) image. Although 3-dimensional SPECT is more accurate than 2-dimensional planar imaging for assessing regional lung deposition patterns, SPECT is limited by the dissociation of ^{99m}Tc from albuterol and the need for longer acquisition times and higher doses of radioactivity to achieve an adequate image (*13,17*).

CONCLUSION

The novel data show that our methodology for radiolabeling monodisperse albuterol particles is robust and that it is possible to achieve good-quality γ-scintigraphy images of in vivo deposition. This unique technique may now be further used to investigate the integral relationship between drug particle-size effects and topographic lung deposition within the human airways and, indeed, determine whether regional airway drug deposition has an important influence on clinical outcome.

ACKNOWLEDGMENTS

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