Effective Penetration of the Lung Periphery Using Radioactive Aerosols:
Concise Communication


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Radioactive microspheres could offer several advantages over gases in the investigation of pulmonary ventilation. Monodisperse microspheres of human serum albumin have been produced using a spinning-disc generator, and kits were prepared for subsequent labeling with technetium-99m. The average labeling efficiency was 88% and unlabeled Tc-99m was removed before aerosol delivery. A simple system was constructed to nebulize and deliver dry monodisperse microspheres. The ventilation images obtained were compared quantitatively with the corresponding krypton-81m images, subdividing the lung regions into inner, central, and peripheral zones. No significant difference was found in the proportions of the total counts for any lung region. There was good agreement between the distributions of microspheres obtained on separate days (r = 0.97, p < 0.001). An "aerosol penetration index" was defined as the ratio of the peripheral to the inner counts for the microspheres normalized by the corresponding ratio for krypton-81m. The mean value of this index for 16 normal subjects was 0.98 ± 0.23 (s.d.), indicating that the microspheres had achieved penetration of the lung periphery. For patients with chronic obstructive lung disease, more localized defects were observed with the microspheres than with krypton-81m. The mean penetration index for this group was only 0.69 ± 0.21 (s.d.). This was significantly different from the value for normal subjects (p < 0.002).


Lung perfusion imaging with radioactive microspheres is well established in the investigation of pulmonary disease. Ventilation studies may also be performed using suitable radioactive gases such as xenon-133, xenon-127, and krypton-81m. During xenon studies performed using the single-breath method, some patients experience difficulty in the detailed breath-holding maneuvers that are required. These difficulties may be overcome by using a washin-washout technique, which requires only tidal breathing throughout the study. One disadvantage of the use of xenon-133 is that the activity administered must be kept low because of the radiation dose from the associated beta emission. This may result in images where the statistical quality is less than desirable. Also the 80-keV gamma energy of xenon-133 results in high absorption losses. The 203-keV gamma energy of xenon-127, and its lack of beta emission, makes it nearly optimal for imaging with modern gamma cameras. This gas, however, is not readily available. Krypton-81m also has the near-optimal energy and results in a low radiation dose to the patient. However, its parent radionuclide (rubidium-81) is cyclotron-produced and has a relatively short half-life (4.7 h), which restricts the general availability of the method.

A method for lung imaging with a technetium-99m aerosol would have the advantage of ready availability and would allow multiple views to be taken. Such a method has been described by Hayes and Taplin (1).
They used a mechanical nebulizer to generate a suspension of polydisperse particles, and removed the larger particles using a "settling bag," resulting in a delivery efficiency of about 10%. Greening et al. (2) used particles nominally less than 2 μm in diameter. These particles were suspended in saline solution and were delivered by the method of Santolicandro et al. (3) using a glass nebulizer. It was not shown by Greening et al. (2) that effective saline droplet evaporation had been achieved before aerosol inhalation. Lewis et al. (4), using laser analysis, showed that the mass median diameter of albumin microspheres nebulized from a saline solution increased from 1 to 12 μm as the relative humidity was increased from 0 to 100%. Even if effective droplet evaporation was achieved, the original labeled particles would still be attached to sodium chloride residues having some unknown size. This might explain the hilar deposits observed by Greening et al. (2) in normal subjects, and also the fact that the lung distribution of their particles was different from that of krypton-81m.

We have developed a simple system for delivering dry monodisperse microspheres of a predetermined size. The pulmonary distribution of microspheres 2 μm in diameter has been compared with that of Kr-81m gas, and the reproducibility of the method has been established.

METHODS

Subjects. A total of 22 subjects were studied; 16 normal subjects (eight nonsmokers, seven asymptomatic smokers, and one asymptomatic exsmoker) and six patients with chronic obstructive pulmonary disease (COPD). Conventional pulmonary function tests were also performed, including the maximum expiratory flow at 25% of forced vital capacity (Vmax25) expressed as a percentage of predicted value. The Vmax25 test is widely considered to be a measure of small-airway obstruction (5) and a wide range of values were observed in the ten asymptomatic smokers (19–99% of Vmax25 predicted). Each subject was fully informed as to the nature of the study, which had the approval of our Divisional Ethical Committee.

Particle production. The pulmonary deposition of particles as a function of particle diameter has been investigated by Stahlhofen et al. (6). In the present study we used microspheres 2 μm in diameter in order to minimize losses in the conducting airways while allowing a reasonably high total deposition to be achieved, predominantly in the respiratory zone. The microspheres were produced from a 0.25% (w/v) solution of human serum albumin using an air-driven spinning top (7). The microspheres were then denatured by heating in olive oil (8) and the unwanted "satellite" particles were removed by centrifuging. The monodisperse microspheres were then washed with ether to remove the oil. Their size range was determined by Coulter counting, and the coefficient of variation was found to be less than 10%.

For a spinning-disc run time of 30 min, this procedure produced 30 mg of albumin microspheres, enough for 125 patient studies. The main stock was kept in the dry state. Sufficient particles for 40 patient doses (16 mg) were added initially to pure ethanol to facilitate suspension; they were then kept in 20 ml of 1% ethanol. This suspension was given a shelf life of 8 wk. Each patient dose of 0.4 mg contained about 75 million microspheres.

Labeling procedure. For each study, 0.5 ml of sodium pertechnetate solution (811 μCi = 30 MBq) was added to a kit containing 0.4 mg of albumin microspheres and 0.01 mg SnCl2·2H2O in 1.0 ml of 25 mM HCl. The development of this kit has been described elsewhere (9). After incubation at room temperature for 20 min, 8.5 ml of 50 mM HCl was added to the vial, which was then centrifuged at 2000 g for 10 min and the supernatant removed. The labeling efficiency was determined by comparing the activities on the microspheres and in the supernatant. The microspheres were then resuspended in 10 ml of ethanol and centrifuged as before. The supernatant was again removed and the Tc99m-labeled microspheres, resuspended in only 0.3 ml ethanol, were used for nebulization.

Delivery system. The delivery system used is shown in Fig. 1. It was constructed as a cube of side 30 cm. The base and three of the sides had 2-mm lead sheet sandwiched between 3-mm Plexiglas sheets to provide radiation protection, the rest being Plexiglas alone. All

FIG. 1. Radioactive aerosol delivery system showing nebulizer at upper right. Respirometer and one-way valve are seen at upper left, with meteorology balloon attached. Three-way valve and low-resistance filter are at the front.
joints were made air-tight and access was provided through a 10-cm threaded porthole on the front side. To minimize impaction losses, the air-jet nebulizer from which the particles were dispersed was located near one corner towards the back of the top side and pointed towards the diagonally opposite corner. It consisted of a 20-gauge hypodermic needle in a brass holder to which a compressed-air supply (15 psi) was connected. A meteorology balloon was suspended from a Plexiglas tube through the top of the box. To achieve a satisfactory resistance at the patient’s mouthpiece, 2.5-cm i.d. connecting tubing was used throughout. A Wright respirometer and one-way low-resistance valve was connected to the balloon. A connection was also made from the respirometer and balloon to one port of the three-way valve. Other parts of this three-way valve were connected to the sealed box, the patient’s mouthpiece, and by a second one-way valve to a low-resistance filter (F, Fig. 2).

The various positions that could be selected at the three-way valve are illustrated in Fig. 2. Position (a) was used to familiarize the patient with mouth breathing while wearing a nose clip. In this position, inspired air was received through the respirometer and the expired air passed through the low-resistance filter. To minimize impaction losses in the major airways and still achieve a high delivery efficiency, patients were instructed to breathe slowly (<0.25 1/sec) during inspiration and to achieve quite large tidal depths (1–1.5 l). Reasonable breathing control could be obtained with simple verbal instructions from the operator, who received constant information on the patient’s breathing pattern from the Wright respirometer. While still in the (a) mode, the Tc-99m-labeled particles were nebulized by application of the compressed-air supply for several seconds. The volatile ethanol quickly evaporated leaving a suspension of dry monodisperse particles. For each study a microscope slide was placed on the base of the delivery system and later removed for inspection by light microscopy. No significant particle aggregation (<1% triplets) was ever observed. The three-way valve was then changed to position (b), allowing the patient to inspire from the sealed box and exhale through the filter, which trapped any exhaled particles. During each inhalation the pressure inside the box was reduced and an equal volume of air from the atmosphere entered the balloon through the respirometer to equalize the pressures. In this mode, the respirometer still provided a measure of breathing pattern. This technique avoids dilution effects and therefore maintains a steady radioactive particle concentration. Aerosol delivery was terminated after approximately 25 l had been removed from the box (balloon close to maximum inflation). The valve was then changed to position (c). In this position the box was closed to the atmosphere, except by way of the filter. Further application of the compressed-air supply collapsed the balloon and flushed residual activity from the box into the filter.

Krypton-81m ventilation images were obtained during tidal breathing from a face-mask connected to a 6.8 mCi (250-MBq) Rb-81→Kr-81m generator supplied with oxygen at 3 l per min.

**Imaging equipment.** Immediately after aerosol administration, posterior images of the lungs were obtained with the subject sitting upright against the high-sensitivity diverging collimator of a gamma camera. An analog image containing 400,000 counts was accumulated in typically 2–3 min, and a 64- by 64-cell digital image was simultaneously accumulated in a computer. The digital image was shown on color and grey-level display systems and was also stored on disc for subsequent analysis. Quantitative comparison of regional deposition was available using a regional selection facility and user-supplied computer programs.

**Radiation dosimetry.** The Administration of Radioactive Substances Advisory Committee (10) gives a dose equivalent of 0.4 rad per mCi of Tc-99m, uniformly distributed within the lungs as macro-aggregated albumin, and since a relatively uniform deposition of microspheres was observed in our study, this figure was used to estimate radiation dose. In the present study we nebulized 0.8 mCi (30 MBq) of Tc-99m microspheres and measured a delivery efficiency of ~40%. This results in a radiation dose of 130 mrad (1.3 mGy) to the lungs for each particle ventilation study. The radiation dose to the lungs from inhalation of Kr-81m has been estimated as only 10 mrad (0.1 mGy) per exposure (11).
RESULTS

The proportions of Tc-99m microspheres and Kr-81m gas in the various lung regions were compared by paired Student's t-test. Comparisons between different groups were made by analysis of variance and unpaired Student's t-test. Values of $p > 0.05$ were considered to be not significant. The average microsphere labeling efficiency, determined over 38 measurements, was 88% ± 4 (s.d.). However, after the labeled particles were resuspended and centrifuged in ethanol, no free Tc-99m was detected. All Tc-99m delivered to the subject's lungs, therefore, was bound to albumin microspheres. The aerosol delivery efficiency, measured using simulated breathing patterns, was 39% ± 2 (s.d.).

Typical microsphere ventilation images and the corresponding Kr-81m images, obtained from a nonsmoker and a smoker, are shown in Fig. 3. The agreement between these images is good and similar results were obtained in all normal subjects, with no evidence of particle impaction in the central airways. To determine whether there was preferential clearance of microspheres from any lung region, five subjects (2 smokers, 3 nonsmokers) were re-imaged one hour after the initial study and the images were subdivided into regions representing the inner, central, and peripheral lung. These regions were chosen to represent 20%, 30%, and 50% respectively of the area of each lung image, and the integrated count within each region was expressed as a proportion of the total counts from both lungs. Although these regions were rather arbitrary, the large airways should predominate in the inner zone and the small airways and alveoli should predominate in the peripheral region. No significant difference ($p > 0.10$) was found between the images acquired one hour apart for any lung region, so images could be obtained from multiple projections up to at least one hour after particle inhalation. The proportions of Tc-99m microspheres and Kr-81m gas observed in the inner, central, and peripheral lung regions are shown in Fig. 4 for all normal subjects. The regions chosen represented 20%, 30%, and 50% respectively of the area of each krypton-81m lung image, and the integrated count within each region was expressed as a proportion of the total count from both lungs. These same regions were then used in the analysis of the aerosol images and were moved on the TV display until the aerosol lung images were placed symmetrically within the outer boundary of the regions. Re-analysis of the krypton images after moving and re-positioning the regions in this way gave a mean difference of 3.4% ± 1.7 (s.d.). Any additional difference between the krypton-81m and aerosol images could be due either to the different emitted energies, or to differences between the deposition of 2-μm particles and the primary inspiratory flow, and possible diffusion, of the gas. Even with different radioactive gases, the detected distribution may vary with gamma energy and physical half-life. For gases with short half-life, the detected distribution will mainly reflect primary arrival, and for gases with long half-lives the distribution may include the effects of diffusion and collateral ventilation. For the nonsmokers the mean percentages of the total lung counts in the inner, central, and peripheral regions were 9.7 ± 1.0 (s.d.), 20.3 ± 1.8, and 20.0 ± 2.3 for the microsphere study, and 9.5 ± 1.0, 19.5 ± 2.0, and 21.0 ± 2.0 for Kr-81m. The corresponding values for the smokers were 9.1 ± 1.0, 19.8 ±

![FIG. 3. Ventilation images from nonsmoking normal subject, obtained from (a) Tc-99m-labeled aerosol, and (b) Kr-81m gas. Aerosol and Kr-81m images from a smoker are shown in (c) and (d) respectively. In both cases, good agreement is observed between aerosol and gas images, with aerosol penetration of lung periphery and no impaction losses in major airways.](image)

![FIG. 4. Comparison of proportions of Tc-99m-labeled microspheres and Kr-81m gas detected from different lung regions. Inner, central, and peripheral regions selected are also shown. Open symbols represent nonsmokers and closed symbols the smokers. Proportions of total lung counts in inner, central, and peripheral lung regions are represented by circles, squares, and triangles respectively.](image)
1.9, and 21.0 ± 2.7 for the microspheres and 9.3 ± 1.0, 19.2 ± 2.4, and 21.5 ± 3.1 for Kr-81m. There was no significant difference between the proportions of microspheres and Kr-81m in any lung region for either group. Similarly there was no difference between either group.

The reproducibility of the technique was assessed by performing repeat studies on seven subjects within one week. The proportion of the total counts in each of six “slices” was determined for each lung. The regions selected and the comparison between the two separate studies are shown in Fig. 5. Good agreement between the two studies was obtained (r = 0.97, p < 0.001). Examples of microsphere and Kr-81m ventilation images obtained from patients with chronic obstructive pulmonary disease are shown in Fig. 6. For all patients with COPD, the microsphere images showed more localized ventilation defects than the Kr-81m images. In some patients more of the lung periphery was visible on the Kr-81m images, indicating that there was reduced penetration of 2-μm particles. The reduced penetration of the lung periphery in the COPD patients may be quantified by defining an aerosol penetration index (API) as the ratio of the counts in the periphery to the counts in the inner region for the Tc-99m microsphere study normalized by the corresponding ratio from the Kr-81m image. This API is similar to that used by Greening et al. (2). The mean value of the API for the combined nonsmoking and smoking group was 0.98 ± 0.23 (s.d.), and for the COPD patients was 0.69 ± 0.21. These values were significantly different (p <0.002).

DISCUSSION

Although lung imaging following inhalation of aerosols was introduced as early as 1965 (12,13), the method has never found general acceptance largely because of technical difficulties associated with its use. The primary technical problem has been excessive deposits within the large conducting airways, which has made image interpretation difficult. Pircher et al. (14) described a delivery system which incorporated a heating chamber to reduce the particle size of a “wet” aerosol by evaporation, while Mullins and Hayes (15) removed large droplets by impaction on baffles. Hayes et al. (16) used a large reservoir bag in the delivery line to remove the larger droplets by preferential settling. Greening et al. (2) used a “wet” delivery method and obtained mean aerosol penetration indices of 0.84 ± 0.14 (s.d.) for normal subjects and 0.44 ± 0.17 (s.d.) for patients with chronic obstructive airflow.

Our results suggest that effective peripheral penetration in normals (asymptomatic smokers and non-smokers) can be achieved using the aerosol technique, but only if the essential conditions of particle size, aerosol monodispersity, and mode of delivery are met. For example it would be quite insufficient to use a polydisperse aerosol having the same average size (mass median aerodynamic diameter) as our particles. The larger particles in the range would deposit by impaction in the conducting airways and give rise to hot spots on the image. We also stress the importance of sizing the actual particles or droplets inhaled by the subject. If the primary particles are delivered via saline solution, it is the size of either the wet droplets or dry bulk residue (including sodium chloride) that must be measured. If the dry bulk residue is collected for sizing, this cannot be satisfactorily accomplished by redispersion in saline solution (2). For aerosol studies where careful control of
particle size is required, we do not recommend the use of saline solution as the dispersal medium. Ethanol provides a suitable alternative and the concentration is kept below about 10 mg/l. In our studies the dispersal of 0.3 ml of ethanol resulted in a concentration of ethanol vapor of 8.5 mg/l. We have found previously that there is no respiratory discomfort or any effect on lung function (as measured by FEV\textsubscript{1} and V\textsubscript{max}50) at this concentration, and that particles dispersed using this technique are delivered in the dry state \((17)\).

Rate and depth of respiration can cause differences in the distribution of aerosols and gases. Milic-Emili et al. \((18)\) studied the effects of different degrees of lung inflation on regional gas distribution, and found differences in basal and apical distribution for different depths of inspiration. However, in terms of a total lung capacity of about 5 l for a normal subject, the difference between the tidal breaths of say 0.7 l used in the krypton studies and the deeper breaths of between 1 l and 1.5 l used for the aerosol studies should be small. Differences in flow rate between the gas and aerosol studies were similarly small. These differences will be minimized when the particle size and breathing pattern for the aerosol inhalation are such that minimal particle losses occur on the conducting airways. Slow respiration increases the mean particle residence time and so enhances peripheral penetration. We found that all subjects were able to follow the simple verbal instructions given during aerosol administration, which typically lasted about 3 min.

In patients with COPD we found incomplete aerosol penetration of the lung periphery, but the differences in the patterns of the aerosol and krypton-81m gas distributions are difficult to explain exclusively in terms of impaction of the particles in constricted central airways. In these patients we did not observe the small "hot spots" previously found by Santolicandro et al. \((3)\) in patients with COPD. Rather we mostly observed diffuse areas of increased aerosol deposition separated by regions of reduced concentration. It is possible that, in addition to any impaction, some degree of collateral ventilation and/or gaseous diffusion may have increased the effective peripheral penetration of Kr-81m beyond the range of the primary inspiratory flow.

For patients without COPD this technique offers a simple and reproducible means of assessing pulmonary ventilation. The delivery system is simple to construct and the method could be performed in any department with conventional nuclear medical facilities. The possible application of the technique to the early detection of small-airways disease is being investigated.

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