A Rapid Analytical Method for Measuring Drug Distribution in Aerosols

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We have developed a rapid analytical method for measuring drug distribution in aerosols. This method utilizes paper chromatography and a cascade impactor system to determine the distribution of nedocromil sodium (Fisons) and a radioactive tracer, technetium-labeled diethylenetriamine pentaacetate (^{99m}Tc-DTPA), in an aerosol generated by a Fisoneb™ nebulizer. Paper chromatography demonstrated that except for Stage 1 of the cascade impactor, which collects the largest particles, more than 93% of the aerosol mixture of nedocromil sodium and ^{99m}Tc-DTPA eluted at the same point. Further, the distribution of ^{99m}Tc-DTPA gamma counts as measured by a cascade impactor and the concentration of nedocromil sodium as measured by densitometry had the same rank order of concentration in the four stages of the cascade impactor. We conclude that this method can rapidly determine the distribution of a drug within a liquid aerosol and validates the potential use of ^{99m}Tc-DTPA as a marker of drug distribution in the respiratory system.

The delivery of drugs to the lungs by aerosol is becoming an acceptable method for drug administration (*1–3*). The increased use of pentamidine in acquired immunodeficiency syndrome (AIDS) therapy (*4*), the development of antiviral drugs for use against respiratory viruses, and the treatment of asthma by aerosol medication emphasize the usefulness of aerosol drugs for the treatment of human disease.

However, many methodological questions are raised by the use of aerosol drugs. Some of these questions concern the amount and pattern of drug deposition in the lungs, the effect of body position and breathing pattern on overall lung distribution of the drug, and the physical characteristics of the drug aerosol. Several investigators have used technetium-labeled diethylenetriamine pentaacetate (^{99m}Tc-DTPA) as a marker of drug deposition in the lungs (*4,5*). However, these studies do not correlate the distribution of ^{99m}Tc-DTPA to drug distribution in the aerosol mixture. Other investigators have used other radioactive markers, bromine-77 (^{77}Br) and ^{99m}Tc-labeled human serum albumin, for drug deposition studies in humans with respiratory disease (*6,7*). However, the ^{77}Br study investigators did not correlate the distribution of ^{77}Br to drug distribution in their aerosol mixture (*6*). Ilowite and coworkers characterized the particle size characteristics of their ^{99m}Tc-labeled human serum albumin-gentamicin aerosol along with saline and ^{99m}Tc, saline alone, and with ^{99m}Tc-labeled human serum albumin alone (*7*). The physical properties of the aerosol were unaffected by the chemical composition of the aerosol solution. However, we do not know the methodology used for these analyses.

Using the drug, nedocromil sodium (disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyranoquinoline-2,8-dicarboxylate, MW = 415), we have developed a rapid method of determining the physical characteristics of an aerosol of this drug, which may be applicable to other drugs as well. The determination of the physical characteristics of drug aerosols would be helpful in advancing the use of this drug delivery method in clinical settings. Thus, the purpose of the present study was to validate our new analytical method of determining drug distribution in an aerosol with the new antiviral drug, nedocromil sodium.

METHODS

Aerosol Delivery and Size Fractionation

A 2-ml (mass of 1.04 mg/ml) ampule of nedocromil sodium 0.5% nebulizer solution was mixed with 1 ml (mass of 5 mg/ml) of 1 mCi ^{99m}Tc-DTPA (physical half-life = 6.02 hr, MW = 492) for a total aerosol volume of 3 ml. The ^{99m}Tc-DTPA was prepared just before each of the three separate trials using a standard DTPA kit (Du Pont, Billerica, MA).

This solution was placed in a Fisoneb™ nebulizer (Rochester, New York) and aerosolized into a 2-l mixing chamber placed directly over a Casella Mark II cascade impactor (London, England). The D_{50} sizes of aerosol particles, which are collected with 50% efficiency, for the four stages of the cascade impactor are 12.4, 3.9, 1.5, and 0.4 μm. The curves

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for Stages 1–3 were obtained from the means of a number of sample, heterogeneous, spray clouds. Curves for Stage 4 were developed by nephelometric measurements using clouds of homogeneous droplets (*8*). We did not measure the aerosol that penetrated the cascade impactor to the back-up filter because this small amount of aerosol would have a particle size less than 0.4 μm, and it is generally accepted that particles below 0.5 μm are not retained in the lungs upon expiration.

The aerosol was delivered to the mixing chamber for 5 min and the flow rate through the cascade impactor was set at 15 l/min. Diluting air was added to the chamber to meet the air flow demand of 15 l/min. At the end of the 5-min aerosolization period, the glass plates attached to each stage of the cascade impactor were removed and gamma counts were recorded by a Ludlum scintillation counter (Model 2200, Sweetwater, TX) attached to a Model 44-2 probe. Three 30-sec counts were recorded for each glass plate, after correction for background counts, and averaged to obtain a mean count. The aerosolized solution of nedocromil sodium ^{99m}Tc-DTPA was then washed off each plate with 8 ml of purified water for analysis by paper chromatography. Subsequent gamma counting of the glass plates showed that recovery of the ^{99m}Tc-DTPA was 95% ± 1.2%.

Paper Chromatography and Optical Densitometry

Triplicate strips of Whatman 31 ET chromatography paper were spotted with one droplet from a precision Eppendorf pipet (mean mass of 13.4 mg ± 0.5, s.e.m.) of the nedocromil sodium ^{99m}Tc-DTPA aerosol mixture from each of the four stages of the cascade impactor according to the standard method of Robbins (*9*). These strips were air-dried for 15 min and then developed in acetone until the solvent front reached a height of 11 cm. The nedocromil sodium remained at the origin, demonstrating that nedocromil sodium was insoluble in acetone. A representative strip was examined and photographed under ultraviolet light at a wavelength of 254 nm. The nedocromil sodium drug was visualized as a fluorescent substance at this particular wavelength. Densitometer readings were performed on the drug spots at each stage of the cascade impactor using a Macbeth TD 504 densitometer (New Burgh, NY).

The radioactivity distribution was obtained by dividing total radioactivity at the origin by the total radioactivity of the paper strip and expressed as a percent. The amount of radioactivity was determined by cutting the strips into 1-cm segments and measuring gamma counts in a sodium iodide well-type scintillation counter.

RESULTS

The radioactivity from each stage of the cascade impactor was calculated as a percent of total counts and plotted on a log-probability scale. The activity median aerodynamic diameter of the aerosol generated from the test solution was 1.4 μm with a geometric standard deviation (GSD) of 1.9. The distribution of radioactivity at each stage of the cascade impactor is shown in Table 1.

Paper chromatography of the two components of our aerosol demonstrated that, except for Stage 1 of the cascade impactor, which collects the largest particles, more than 93% of the mixture eluted at the same point. Figure 1 shows the aerosol mixture photographed under ultraviolet light at each of the four stages of the cascade impactor for one trial. Densitometer readings of the negatives of the photographs in Figure 1 demonstrate a rapid method for determining the concentration of our aerosol mixture at the various stages of the cascade impactor (Table 2). Most of the aerosol mixture, more than 93%, was deposited on Stages 3 and 4 as demonstrated by the cascade impactor profile. In addition, the ratio of ^{99m}Tc-DTPA counts from Stage 4 to Stage 3 was 1.36 (Table 1), while the densitometer ratio from Stage 4 to Stage 3 was 1.10 (Table 2).

TABLE 1. ^{99m}Tc-DTPA Counts at Each Stage of the Cascade Impactor

Stage	Counts*	% of Total Counts
1	8,841 (±2,003)	1.1
2	43,952 (±14,540)	5.6
3	313,279 (±14,971)	39.6
4	425,174 (±48,255)	53.7

* The counts are a mean (±s.e.m.) of three trials.

DISCUSSION

We have demonstrated a rapid method that is useful for determining the distribution of the drug, nedocromil sodium,

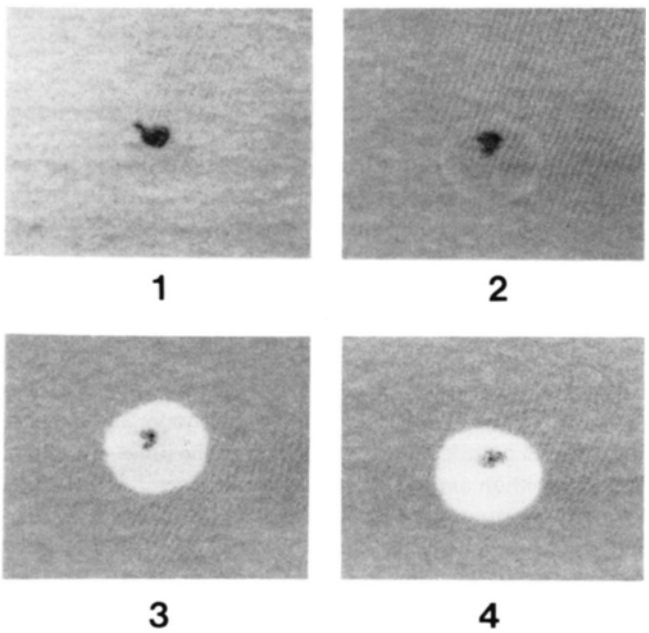

FIG. 1. Photographs of nedocromil sodium ^{99m}Tc-DTPA aerosol mixture eluted by paper chromatography and photographed under ultraviolet light at a wavelength of 254 nm. The numbers correspond to the four stages of the cascade impactor. The black dot in each photograph represents the origin.

TABLE 2. Densitometer Readings of Nedocromil Sodium ^{99m}Tc-DTPA Aerosol Mixture from Negatives of Paper Chromatography Photographs

Stage	% Radioactivity at Drug Spot	Densitometer Reading
1	65.8 (21.2)	0.85
2	93.7 (4.1)	0.86
3	98.8 (0.8)	1.00
4	95.4 (3.6)	1.10

The densitometer reading is in densitometer units. The % radioactivity is a mean (± s.e.m.) of three trials. 99.9% of the ^{99m}Tc-DTPA standard was found at the origin.

in an aerosol. More than 93% of the aerosol mixture was deposited on Stages 3 and 4 as demonstrated by the cascade impactor profile. Both the cascade impactor and densitometer ratios of Stage 4 to Stage 3 were similar, 1.36 and 1.10, respectively. However, the ^{99m}Tc-DTPA cascade impactor/nedocromil sodium densitometer ratios did not correlate as well at the larger particle sizes of Stage 1 (12.4 μm D_{50}) or Stage 2 (3.0 μm D_{50}). It is not likely that these large particles would be deposited in the peripheral areas of the lungs, where, as we have demonstrated, most of the permanent injury induced by a respiratory virus occurs (*10*). The ^{99m}Tc-DTPA at Stages 2, 3, and 4 of the cascade impactor did not separate from the nedocromil sodium drug (Table 2). However, at the largest particle size, Stage 1, only 65.8% of the ^{99m}Tc-DTPA was associated with nedocromil sodium. Thus, we have shown that ^{99m}Tc-DTPA stays with the nedocromil sodium and thus may be utilized as a marker of deposition of this drug in the respiratory system, at particle sizes below 4 μm.

We also attempted to determine the ^{99m}Tc-DTPA nedocromil sodium aerosol distribution by means of a high pressure liquid chromatography (HPLC) system equipped with a multiple wavelength spectrophotometer. However, the HPLC data did not correlate as well as paper chromatography with the radioactivity distribution determined by the cascade impactor. Furthermore, HPLC is expensive (start-up costs for this experiment were in excess of $1,000) and time-consuming (this experiment took greater than 12 hr of run time). Consequently, our paper chromatography method has the advantages of greater accuracy and rapidity (less than two hr) and lower expense than the HPLC method (total cost of paper chromatography method is about $100).

We have determined the distribution of nedocromil sodium using a Fisoneb™ aerosol system. Aerosols with a GSD of greater than 1.22 are generally termed "heterodisperse" (*11*) and because the GSD of our aerosol was greater than 1.22, we must classify our aerosol mixture as heterodisperse. However, one might expect the distribution of nedocromil sodium to change with a different nebulizer system.

We postulate that a goal of any drug aerosol system would be to create a heterodisperse aerosol as determined by a GSD greater than 1.22. Two of the major factors that determine aerosol deposition in the lungs are particle size and inhalation flow. Inhalation flow can be variable, especially in patients with severe obstructive lung disease. Thus, a heterodisperse aerosol would significantly influence the deposition pattern of the drug in the lungs, especially in patients with severe lung disease. Since, on average only 10% of a therapeutic aerosol dose actually reaches the lungs (*12*), changing the particle size characteristics of the drug aerosol by utilizing different nebulizers may be beneficial in attempting to target therapeutic agents to specific sites in the lungs.

The similar molecular weights of ^{99m}Tc-DTPA and nedocromil sodium, 492 and 415, respectively, were possibly a factor for the similar aerosol distribution profiles in our study. However, other radioactive tracers, indium-111 (^{111}In) transferrin (MW = 76,111), gallium-67 (^{67}Ga) desferoxamine (MW = 624), ^{111}In-DTPA (MW = 545), and ^{99m}Tc-DTPA compounds such as cytochrome c (MW = 12,500), myoglobin (MW = 17,000), and albumin (MW = 68,600) have been developed and used to measure pulmonary clearance (*13,14*). Consequently, it may be possible to match the molecular weight of the drug to be studied with a radioactive tracer of similar molecular weight. However, we do not have any direct evidence that matching radioactive tracer weights with drug weights will give a similar aerosol distribution profile and the validity of this theory awaits further study.

In summary, we have developed and applied a rapid method for characterizing the distribution of a drug in an aerosol mixture. We conclude that ^{99m}Tc-DTPA and nedocromil sodium are distributed among the cascade impactor size categories in a similar rank order. Consequently, we validate the use of ^{99m}Tc-DTPA as a potential marker of drug deposition in the respiratory system.

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