Scintigraphic Assessment of Deposition of Radiolabeled Fluticasone Delivered from a Nebulizer and Metered Dose Inhaler in 10 Healthy Dogs

K.E. Chow [iD], D. Tyrrell, M. Yang, L.A. Abraham, G.A. Anderson, and C.S. Mansfield

Background: Aerosolized medications are increasingly being used to treat respiratory diseases in dogs. No previous studies assessing respiratory tract deposition of radiolabeled aerosols have been performed in conscious dogs.

Hypothesis/Objectives: Assess respiratory tract deposition of radiolabeled, inhalant corticosteroid (fluticasone propionate labeled with ^{99m}Tc) delivered from a nebulizer and metered dose inhaler (MDI) to healthy dogs.

Animals: Ten healthy Foxhounds.

Methods: Prospective, randomized, cross-over pilot study. Initial inhalation method (nebulizer or MDI) was randomly assigned. Treatments were crossed over after a 7-day washout period. Treatments initially were performed using sedation. Dogs were imaged using 2-dimensional planar scintigraphy, with respiratory tract deposition quantified by manual region-of-interest analysis. Deposition calculated as percentage of delivered dose. Six of 10 dogs were randomly selected and reassessed without sedation.

Results: Inhalation method had significant effect on respiratory tract deposition ($P = 0.027$). Higher deposition was achieved by nebulization with mean deposition of 4.2% (standard deviation [SD], 1.4%; range, 1.9–6.1%); whereas MDI treatment achieved a mean of 2.3% (SD, 1.4%; range, 0.2–4.2%). Nebulization achieved higher respiratory tract deposition than MDI in 7 of 10 dogs. No statistical difference ($P = 0.68$) was found between mean respiratory tract deposition achieved in dogs when unsedated (3.8%; SD, 1.5%) or sedated (3.6%; SD, 1.7%).

Conclusions and Clinical Importance: Study confirms respiratory tract deposition of inhalant medications delivered from a nebulizer and MDI in healthy dogs, breathing tidally with and without sedation. Respiratory tract deposition in these dogs was low compared to reported deposition in adult humans, but similar to reported deposition in children.

Key words: Aerosol treatment; Respiratory tract deposition; Nebulizer; Metered dose inhaler; Dogs.

Modern aerosol devices commonly used in people for treatment of respiratory diseases include metered dose inhalers (MDI), dry powder inhalers (DPI), and nebulizers.[1,2] The use of aerosolized corticosteroids and bronchodilators delivered from a MDI, spacer, and face mask apparatus has been reported in cats with lower airway disease and in dogs with chronic bronchitis and eosinophilic bronchopneumopathy.[3,4] In

Abbreviations:

γ	gamma
2D/3D	2 or 3 dimensional
99mTc	technetium-99m
ACF	attenuation correction factor
CI	confidence interval
CPM	counts per minute
DTPA	diethylenetriamine pentaacetic acid
DPI	dry powder inhaler
HPLC	high-performance liquid chromatography
MDI	metered dose inhaler
MMAD	mass median aerosol diameter
Na^{99m}TcO$_4$	sodium pertechnetate
ROI	region of interest

both dogs and cats, aerosols also can be administered by use of an ultrasonic or compressed air nebulizer.[5] Dry powder inhalers require deep inhalations to trigger the device and, as a result, have not been adapted for use in animals.

Evaluation of aerosol drug delivery can be performed by assessment of total and regional respiratory tract deposition which can be achieved by adding a γ-emitting radionuclide to the drug formulation under analysis.[6,7] Once administered, the radionuclide can be mapped by 2-dimensional (2D) or 3-dimensional (3D) gamma scintigraphic imaging.[6] Before scintigraphic imaging, validation must be performed to ensure the radionuclide is an adequate marker for the drug and that the radiolabeling procedures do not alter the particle size distribution (PSD) of the drug.[6,7]

Despite the increasing use of aerosol therapies in small animals, there are few scientific studies assessing

their efficacy. Studies assessing deposition of aerosol therapies in conscious small animals are limited to a single report describing scintigraphic imaging of nebulized [99m]technetium (^{99m}Tc) delivered via a spacer and face mask apparatus in conscious cats.[8] Reports of scintigraphic assessment of aerosol deposition in dogs have been restricted to research studies in the medical field in which anesthetized and intubated dogs are used as models for people.[9–11] To date, there are no scintigraphic studies investigating deposition patterns of radiolabeled drugs administered by aerosol devices in conscious, sedated, or unsedated dogs.

The objectives of our pilot study were to qualitatively and quantitatively assess and compare the deposition of aerosolized fluticasone propionate administered to healthy, conscious dogs via a nebulizer or MDI, using radiolabeling and 2D planar gamma scintigraphy. The working hypothesis was that the 2 devices would not differ in total or regional deposition.

Materials and Methods

Animals and Study Design

This study was a prospective, randomized, crossover pilot study using 10 healthy client-owned foxhounds. Approval was granted by the University of Melbourne Animal Ethics Committee (AEC ID 1112263.2). The foxhounds ranged from 2 to 6 years of age and weighed between 28 and 37 kg. All dogs were up-to-date with vaccinations, worming prophylaxis, and had shown no clinical signs of respiratory disease (eg, coughing) for more than 4 weeks before the start of the study. All 10 dogs underwent complete physical examinations and 2-view thoracic radiography to exclude the possibility of underlying respiratory disease as far as possible. Each dog underwent 2 individual, short training sessions in which they were acclimated to the face mask and spacer apparatus to the point where they did not resist mask placement; during this time, they also were exposed to the noise of an operating nebulizer. The dogs were randomly assigned to 2 groups by lottery without replacement, determining the sequence of treatments. The dogs were given each of the 2 treatments separated by a 7-day washout period. For the initial study, dogs were given acepromazine 0.05 mg/kg IV and were mildly sedated but conscious during aerosol inhalation from both devices. Subsequently, a second study was performed in which 6 of the dogs were selected randomly and received aerosol administration without sedation. These 6 dogs received an additional 5 training sessions to acclimatize them to the nebulizer and MDI.

Radiolabeling and Inhalation Procedures for Nebulization

Commercially available liquid nebules containing fluticasone propionate[a] and a jet nebulizer[b] were sourced for this part of the study. The radiopharmaceutical used was [99m]technetium-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) which was manually mixed with aqueous fluticasone propionate from the nebules and the mixture nebulized as described previously.[12] Each nebulizer bowl was filled with a 1.5-mL mixture containing 100 MBq of ^{99m}Tc-DTPA and 200 μg of fluticasone propionate. The radioactive dose in the nebulizer bowl was measured in a dose calibrator[c] before and after aerosol administration. The nebulizer bowl was connected to a baffle, a right-angled connector and a face mask; the face mask was placed snugly onto the dog's muzzle. All face masks were detergent-coated to decrease electrostatic charge before

use. Each dog inhaled from the operating nebulizer for a 1-minute period during which the dog was allowed to breathe tidally.

Radiolabeling and Inhalation Procedures for the MDI

Commercially available MDIs containing fluticasone propionate were sourced; the radiopharmaceutical used was sodium pertechnetate (Na[99m]TcO$_4$). The techniques employed for radiolabeling have been reported previously.[13,14] Briefly, sodium pertechnetate was sonicated with chloroform, and the mixture passed through a phase separation filter into an empty canister. The chloroform subsequently was evaporated from the canister under a stream of nitrogen gas, leaving a ^{99m}Tc-lined canister. Both the radioactive canister and the commercial canister of fluticasone propionate were supercooled with dry ice. The commercial canister containing fluticasone propionate was decrimped, and the contents poured into the ^{99m}Tc-lined canister. The radioactive canister containing fluticasone then was recrimped. After undergoing validation procedures, this canister was connected to a spacer[d] and a face mask for aerosol administration. Each dog was given 2 actuations from the MDI; after each actuation into the spacer, the dog was allowed 5 tidal breaths. Radioactivity in the canister was recorded with a dose calibrator before and after actuation. Before inhalation, all face masks and spacers were detergent-coated to decrease electrostatic charge.

Validation of Radiolabeling for MDIs

Validation procedures were performed to ensure that radiolabeling of the MDI did not change the product characteristics; these techniques have been described previously in in vitro studies and in studies on adult human males.[14] Briefly, the PSD of the commercial canister and the radioactive canister was measured with a Marple-Miller Cascade Impactor;[e] the results were compared to ensure no alteration to the PSD by the radiolabeling procedures. The Marple-Miller Cascade Impactor is a 5-stage apparatus used to assess PSD of aerosols in a moving airstream. It consists of a mouth piece that allows the MDI to be attached, followed by a "throat" stage that mimics the human throat, and 5 stages of impaction starting at 10.0, 5.0, 2.5, 1.25, and 0.625 μm followed by a filter as the last stage. Each stage collects particles larger than the indicated size but smaller than the previous stage. Samples collected at each stage can be extracted for chemical analyses. High-performance liquid chromatography was used to assess the amount of fluticasone propionate delivered per actuation to ensure no clinically relevant change had occurred to the total mass delivered per actuation after radiolabeling.

Imaging

After aerosol inhalation, 2D planar scintigraphy with a single-headed gamma camera[f] immediately was performed. Two-minute acquisitions of the following views were acquired in the following order: ventral chest, ventral abdomen, dorsal chest, dorsal abdomen, lateral head and neck (right), lateral head and neck (left), and equipment. The period of time between administration of aerosol treatment and end of image acquisition of all regions ranged from 15 to 22 minutes for 19 of 20 studies. For the remaining study, the period of time between aerosol treatment and end of image acquisition was 29 minutes.

Transmission Scanning

A flood source was used to measure tissue attenuation of radioactivity for each dog. Briefly, a rectangular flood phantom[g]

was sourced, and ^{99m}Tc was placed inside the phantom as described previously.[15] A uniformity source image was acquired with the phantom placed a known distance from the gamma camera. The dog then was placed between the flood source and the gamma camera, and transmission images were acquired of the thorax, abdomen, and lateral head. Regions of interest (ROI) were drawn around the head, lungs and stomach, and the count rates determined (N_T). These were placed onto the uniformity source image and the count rate determined (N_O). Using this data, an attenuation correction factor (ACF) was calculated for each dog and each region using the following formula:[15]

$$ACF = \left(\frac{N_o}{N_T}\right)^{1/2}$$

Calculation of Regional Deposition

Deposition in each region (equipment, head, lungs, esophagus, stomach, urinary tract) was quantified by manual ROI analysis. All small, focal, and intense pools of radioactivity within the region of the mediastinum were recorded as originating from the esophagus (not the trachea). The investigators made this assumption based on the subjectively intense but focal nature of these pools of radioactivity, mimicking the appearance of the swallowed radioactivity in the stomach. These pools of radioactivity were unlikely to represent inhaled radioactivity (such as that seen within the region of the lungs) because inhaled activity was subjectively more diffusely distributed.

Deposition in counts per minute (cpm) was recorded for each region, and the geometric means were calculated by averaging counts from the dorsal and ventral or left and right views. Adjustments were made for background activity, length of acquisition, delay of time of acquisition from administration (radioactive decay), and tissue attenuation (ACF). Respiratory tract deposition finally was calculated as a percentage of the delivered dose of radiolabeled aerosol in cpm.

Statistical Analysis

The following response variables were calculated for each dog as a percentage of the delivered dose: dose recovery, respiratory tract deposition, head and gastrointestinal tract deposition, and equipment deposition. The mean and standard deviation (SD) of the above variables were calculated for each treatment group. An analysis of variance with effects of sequence, dog within sequence, period, and treatment was conducted to compare the 2 devices. A normal distribution of the residuals was assessed by the Shapiro-Wilk test, and these did not show evidence of non-normality because all 4 P-values were >0.05. The correlation coefficient between delivered dose (cpm) and respiratory tract deposition (cpm) was calculated for each of the 2 devices using the same 10 dogs. These 2 dependent correlations with nonoverlapping variables then were compared by the *cocor* package.[h] The assumption of normality of both variables was satisfied by the Shapiro-Wilk test. Six of the 10 dogs then were selected randomly and reassessed without sedation after additional training (3 inhaled from the nebulizer, 3 from the MDI). A paired t-test was used to assess for differences in respiratory tract deposition in the 6 dogs with and without sedation (while inhaling from the same device). Statistical significance was set at $P < 0.05$, and Stata Version 14.2 software[i] was used for analyses, except for comparison of correlation coefficients.

Results

Animals

All dogs were normal on physical examination with no history of respiratory signs for >4 weeks before the study (including coughing, dyspnea, tachypnea, or expectoration). All thoracic radiographs were assessed by a resident in veterinary radiology under supervision by radiologists; these were assessed to be normal with a thymic remnant observed in 1 dog.

Validation of Radiolabelling

In vitro results confirmed no significant alteration to the PSD of the commercial preparation of fluticasone propionate by the radiolabeling process (Fig 1). Measurement of radioactivity at each stage of the Marple-Miller Impactor also confirmed that the radiolabel was a suitable marker for the drug (Fig 1). High-performance liquid chromatography confirmed the amount of fluticasone propionate per actuation was not altered by the radiolabeling process. The majority of aerosol particles of the commercial preparation and the radiolabeled preparation were deposited within the device (the actuator of the MDI), the "throat" of the Marple-Miller Impactor, and between the 1.25 and 5 μm stages (Fig 1). The mass median aerosol diameter (MMAD) of the commercial preparation of fluticasone propionate was 3 μm; the MMAD of the radiolabeled preparation of fluticasone propionate was 2.7 μm. The PSD of the nebulizer used in the study is reported by the manufacturer at a MMAD of 1.52 μm. The particles for both devices are likely in the "respirable fraction" (the mass fraction of inhaled particles that will penetrate to unciliated airways) for dogs according to experimental studies performed in Beagles.[16]

Dose Administered Versus Counts Retrieved

Using the nebulizer, the dogs were given between 3.0 and 16.6 MBq of radioactivity at each treatment. Using the MDI, the dogs were given between 23 and 60 MBq of radioactivity at each treatment. Dose recovery was highly variable, for nebulization between 57 and 88% and for the MDI between 28 and 61% of the administered dose was recovered in counts. Higher recovery was achieved by nebulization with mean recovery of 69.0% (SD, 9.4%), whereas MDI treatment achieved a mean of 44.5% (SD, 11.2%). The difference in mean recovery was 24.5% (95% CI, 15.3 to 33.7%; $P = 0.0003$). A significant correlation was found between the delivered dose (converted to cpm) and respiratory tract deposition (cpm) for nebulization ($r = 0.71$; 95% CI, 0.14 to 0.92; $P = 0.022$); the correlation was not significant for the MDI ($r = 0.26$; 95% CI, −0.44 to 0.77; $P = 0.46$; Fig 2A,B), but the difference between these 2 correlation coefficients was not significant ($P = 0.23$).
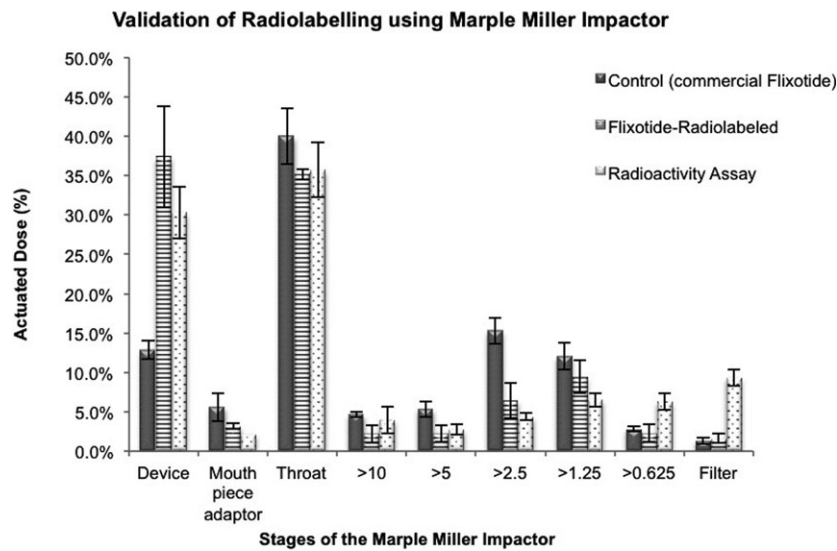
Validation of Radiolabelling using Marple Miller Impactor

Fig 1. Particle size distributions of Flixotide before radiolabeling (Control), after radiolabeling (Flixotide-Radiolabeled), and amount of radioactivity (Radioactivity assay) collected at each stage of the Marple-Miller Impactor (the 5 stages between the "throat" and "filter" are indicated by particle sizes in microns or μm). The actuated dose (%) is presented with error bars indicating the 95% confidence interval at each stage of the Impactor. It can be appreciated that the radiolabeled preparation had a similar particle size distribution to the control preparation.

Respiratory Tract Deposition

Quantitatively, nebulization achieved higher respiratory tract deposition percentage in 7 of 10 dogs and MDI treatment achieved higher respiratory tract deposition percentage in 3 of 10 dogs (Fig 3). Treatment (nebulization versus MDI) had a significant effect on respiratory tract deposition ($P = 0.027$). Higher deposition was achieved by nebulization with mean deposition of 4.2% (SD, 1.4%; range, 1.9–6.1%), whereas MDI treatment achieved a mean of 2.3% (SD, 1.4%; range, 0.2–4.2%). The difference in mean deposition was 1.9% (95% CI, 0.3 to 3.6%) between the 2 treatments.

Qualitatively, there was evidence of deposition of radiopharmaceutical in both the central and peripheral parts of the lungs with both devices (Figs 4 and 5). Respiratory tract deposition was subjectively more uniform among the 10 dogs when they inhaled from the nebulizer (Fig 4) and subjectively more variable among the 10 dogs when they inhaled from the MDI (Fig 5). In 1 particular dog, we observed very poor compliance during administration of aerosol from the MDI (resistance to mask placement and frequent movement); this dog had the lowest respiratory tract deposition of all dogs in the study, with respiratory tract deposition of 0.2% of dose delivered (results from this dog were included in statistical analyses because Grubb's test for an outlier was not significant).

Extrathoracic Deposition

Deposition in the head and gastrointestinal tract (muzzle, oropharynx, esophagus, and stomach) was calculated for all 10 dogs and compared between the 2 groups. The nebulizer achieved a mean deposition of 50.0% (SD, 8.8%; range, 37.6–64.8%), and the MDI achieved a mean deposition of 5.3% (SD, 2.5%; range, 1.6–10.0%) of retrieved counts in the head and gastrointestinal tract. The difference of 44.7% (95% CI, 39.6 to 49.8%) between the 2 groups was statistically significant ($P < 0.0001$). Deposition in the equipment (face mask and connectors for the nebulizer; actuator, spacer, face mask, and connectors for the MDI) was calculated for all 10 dogs and compared between the 2 groups. The nebulizer achieved a mean deposition of 13.9% (SD, 4.9%; range, 9.6–24.5%), and the MDI achieved a mean deposition of 36.9% (SD, 9.2%; range, 24.5–50.3%) of retrieved counts in the equipment. The difference of −23.0% (95% CI, −31.2 to −14.8%) was statistically significant ($P = 0.0002$).

Unsedated Versus Sedated Treatment Administration

Analyses of respiratory tract deposition in the 6 dogs undergoing a second treatment without sedation indicated no statistical difference ($P = 0.68$) between mean respiratory tract deposition achieved when the dogs were unsedated and with the same device (mean, 3.8%; SD, 1.5%) and deposition achieved by the same dogs with sedation (mean, 3.6%; SD, 1.7%; Fig 6). The individual differences between unsedated and sedated values varied among dogs from −1.2% to 1.5%. The mean difference was 0.2% (95% CI, −0.9 to 1.3%).

Discussion

Our pilot study is the first to qualitatively and quantitatively investigate the aerosol deposition patterns of a nebulizer and MDI in sedated and unsedated, healthy dogs. Our results show that both devices can achieve
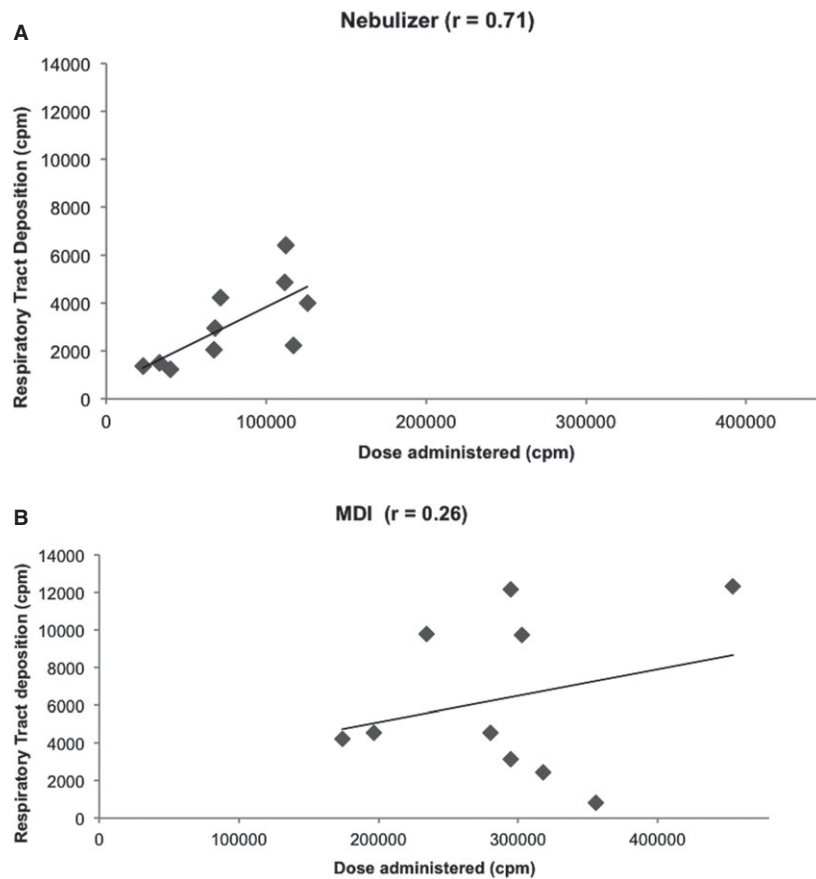
Fig 2. (A, B) Scatterplots showing the correlation between the dose administered in counts per minute (x-axis) and the respiratory tract deposition in counts per minute (y-axis) from each of the 10 healthy dogs during nebulization (A) and during use of a MDI (B). Note the linear correlation ($r = 0.71$, $P = 0.022$) in counts per minute during nebulization and lack of a linear correlation ($r = 0.26$, $P = 0.46$) in counts per minute during use of a MDI.

deposition of radiolabeled fluticasone propionate in the lungs of healthy dogs. The null hypothesis was disproven with nebulization achieving higher respiratory tract deposition than the MDI by approximately 2% of dose delivered. Similarly, the extrathoracic deposition patterns also were statistically different with the nebulizer achieving significantly higher deposition in the head and gastrointestinal tract of the dogs and the MDI achieving higher deposition in the equipment.

Respiratory tract deposition from both devices in this group of dogs was generally low, ranging from 0.2 to 6.1% of dose delivered from both devices. This percentage is very low compared to that achieved in people, with contemporary studies reporting between 40 and 50% respiratory tract deposition in adults.[2] However, this level of respiratory tract deposition is similar to that achieved in infants and toddlers under the age of 5; respiratory tract depositions of 2% from 1 study of children <5 years with obstructive airway disease using a MDI[17] and another of approximately 5% deposition from a study of children from 2 to 4 years of age with stable asthma using both a nebulizer and a MDI have been reported.[12] The major postulated reason for the inefficiency of aerosol systems in children <5 years of
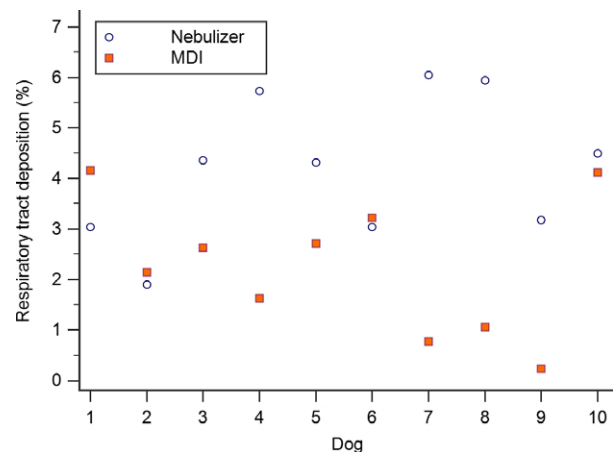

Fig 3. Respiratory tract deposition for each device in 10 healthy foxhounds when they inhaled from the nebulizer and when they inhaled from the MDI. The nebulizer mean was significantly greater than the MDI mean. The mean difference was 1.9% (95% CI 0.3 to 3.6, $P = 0.027$).

age is the requirement for tidal breathing during aerosol administration, as a result of their inability to perform forceful and prolonged inhalations or breath-holds.[18] It
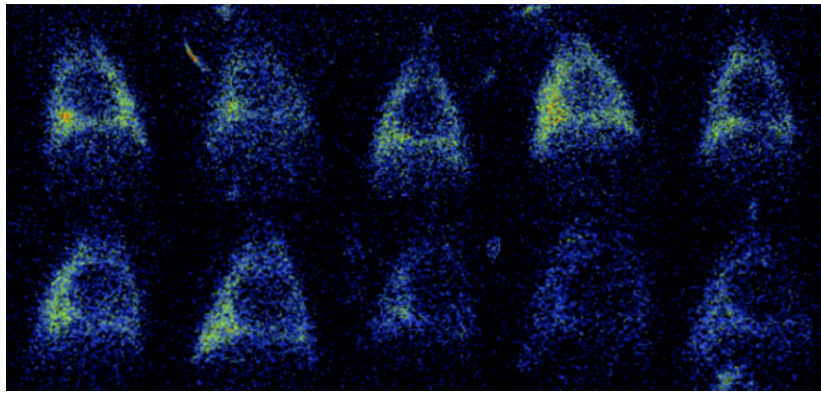
Fig 4. Ventrodorsal 2D scintigraphic images of the 10 dogs when they inhaled from the nebulizer. Subjectively, there is both peripheral and central respiratory tract deposition, and deposition is more uniform among the dogs in this group.
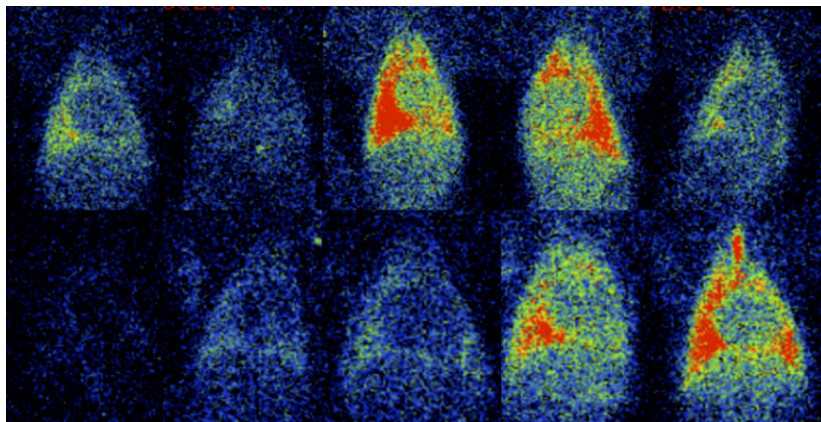

Fig 5. Ventrodorsal 2D scintigraphic images of the 10 dogs when they inhaled from the metered dose inhaler. Subjectively, there is both peripheral and central respiratory tract deposition, and deposition is more variable among the dogs in this group with some dogs achieving large amounts of deposition (red or "hot" regions) and some dogs achieving small amounts of deposition (blue, black or "cold" regions).
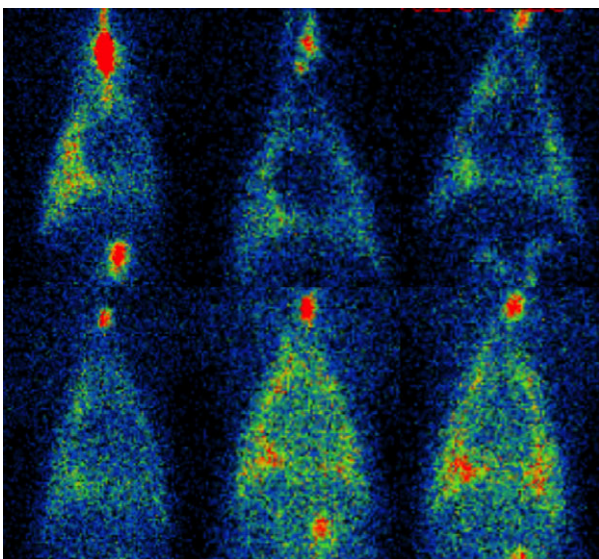

Fig 6. Ventrodorsal 2D scintigraphic images of the 6 unsedated dogs, inhaling from the nebulizer (top row) and from the MDI (bottom row).

is likely that this is the same reason for the inefficiency of aerosol systems in achieving respiratory tract deposition in animals, which are also limited to tidal breathing for aerosol administration.

In our study, nebulization achieved higher mean respiratory tract deposition by approximately 2% of dose delivered when compared to the MDI. The medical literature contains studies that show the devices are equally effective in treatment of pediatric patients with acute asthma,[12,19,20] and studies that show a statistical difference between the devices.[21–23] In 3 studies that showed a difference between the devices, all showed the MDI attached to a spacer to be superior to nebulization in achieving improved indices of respiratory function after administration of bronchodilators in children with acute asthma.[21–23] However, there is evidence in the medical literature that suggests deposition patterns of aerosols delivered from nebulizers may be different from those delivered from MDIs and DPIs.[24] Improved symptom control has been shown in cases of cough-associated asthma when adult patients were switched to nebulization as a predominant form of treatment. The postulated reason for this improvement is the

movement of particles during all phases of the respiratory cycle (leading to increased deposition in larger airways), with possible influences from the airway humidification achieved during nebulization and the slower breathing patterns employed.[24] Postulated reasons for the difference in performance of the devices between children and dogs may include the following: differences between performance of the devices between different species; use of varying indices of performance, as our study investigated imaging results rather than clinical indices in respiratory function; and differences in performance of the devices in a population affected by asthma rather than a healthy population such as the dogs used in our study. For our study, the major postulated reason for the superior performance of the nebulizer is the continuous nature of aerosol treatment with nebulization, versus the momentary pulses of treatment delivered by the MDI; non-compliance then may result in unsuccessful administration of an entire dose of aerosol from the MDI. This hypothesis is supported by the statistically significant correlation between the delivered dose of aerosol and respiratory tract deposition from the nebulizer, suggesting that nebulization is a reliable way to deliver aerosol to dogs. Additionally, there is more equipment between the MDI and the animal in comparison with the nebulizer and the animal; this results in a greater opportunity for leakage of aerosol between the components if the seals between them are not complete.

Qualitative assessment of the respiratory tract deposition images showed that radiolabeled fluticasone propionate reached the periphery of the lung fields, suggesting deposition both in the central and peripheral airways. This observation does not, however, prove deposition in smaller airways because research in the human medical field shows that even quantitative calculation of peripheral versus central lung deposition based on 2D images is a crude indicator of small airway deposition, predominantly because of overlapping of alveolar and airway structures, and overlapping of airways in the hilar region of lungs in people.[6] More accurate calculations and ratios can be made by 3D imaging such as single-photon emission computerized tomography or positron emission tomography.[6] These methods were not available in our study.

Our study showed nebulization resulted in higher deposition in the head and gastrointestinal tract of dogs (including the muzzle, oropharynx, esophagus, and stomach) and the MDI resulted in higher deposition in the equipment (including the actuator, spacer, and face mask). These results are not surprising because 1 purpose of the spacer is to allow larger particles (which typically deposit in the oropharynx) to impact on the inner walls of the device, leaving a fine aerosol available for inhalation.[25] Because a spacer is not used in nebulization, the larger particles are likely to have been delivered to the dogs' muzzle or oropharynx and subsequently swallowed. Although serum concentrations of fluticasone propionate were not measured in the dogs in our study, nebulization potentially could result in higher systemic absorption and unwanted adverse effects

because of a higher level of deposition on the head and in the gastrointestinal tract. Fluticasone propionate delivered via a MDI and spacer resulted in mild suppression of the hypothalamic-pituitary-adrenal axis (HPAA) in dogs.[26] Depending on the metabolism of the corticosteroid aerosolized, nebulization of a steroid could result in more suppression of the HPAA and clinical signs of polydipsia, polyuria, or polyphagia in dogs. For the purposes of our study, all of the spacers and face masks used were washed with detergent and left to dry without rinsing to decrease electrostatic charge. Electrostatic charge has been shown to result in significant reduction in aerosol delivery through a spacer in an in vitro setting, stressing the importance of equipment preparation before use.[27]

No statistically detectable differences were shown between the sedated and unsedated groups of dogs, with mean respiratory tract deposition of both groups being approximately 3.5–4.0%. The reason for the use of sedation in the initial study was the unforeseen reaction of the dogs to the odor of the aerosol plumes, to which they were not acclimated, having been exposed only to the face mask apparatus and the noise of an operating nebulizer. Subsequently 6 of the dogs were retrained using the same medications as employed in the study. Subjectively, we observed better compliance in the group of unsedated dogs after retraining, but this difference was not expressed in any differences in respiratory tract deposition between the 2 groups. This observation may be explained by the use of sedation in the first group, resulting in similar levels of compliance between the sedated and unsedated dogs, or, less likely, that compliance does not equate with better respiratory tract deposition in dogs. In 1 particular dog in our study, we observed high levels of resistance to mask placement and frequent movement during treatment. This dog had the lowest respiratory tract deposition of all dogs in the study, with a respiratory tract deposition of 0.2% of dose delivered. In pediatric patients, a leak in the face mask has been shown to greatly decrease drug delivery to the patient[28]; it is presumed that a tightly fitting mask is equally important in aerosol delivery to veterinary patients. Poor compliance and patient movement are likely to substantially affect the airtight seal achieved in a well-fitted mask, resulting in loss of aerosol to the environment and decreased respiratory tract deposition. Measurement of exhaled and leaked aerosol in future studies will facilitate understanding where and why the majority of loss is occurring during aerosol administration to veterinary patients.

Despite the finding that nebulization achieved a higher mean respiratory tract deposition percentage in this group of healthy dogs, a number of other factors will influence the choice of device for each patient. Additionally, 3 of the 10 dogs in our study achieved higher respiratory tract deposition percentage with the MDI than with the nebulizer, and it should be reiterated that individuals can respond better with 1 particular device than the other. The factors to take into account include the following: potential for higher systemic absorption during nebulization; ability to mix

medications for aerosol administration in nebulization that cannot be performed using a MDI; compliance or resistance to a particular device; costs of equipment or medications; time required for aerosol administration, which is longer for nebulization; and reliability of nebulization for delivery of aerosol, particularly for noncompliant patients. Based on our results, potential ways to manage poor clinical response to aerosol treatment in practice may include the following: increasing doses of medications delivered, improving seal of face masks, detergent coating of spacers and face masks, or switching devices for administration of aerosol if an animal is noncompliant for a particular device.

Limitations of our study include the small sample size, use of healthy dogs, and the use of 1 particular breed, resulting in a relatively homogenous population. The crossover nature of the study did eliminate animal factors as a source of variation between the 2 groups, but the findings cannot be extrapolated to other breeds or dogs with respiratory disease. Another major limitation was the lack of access to pneumotachometry at the time of the study. As a result, no comparisons of aerosol deposition in the respiratory tract to the number or volume of each dog's breaths could be performed. Lastly, the assumption that mediastinal radioactivity originated from the esophagus rather than the airways may have introduced error into the calculation of respiratory tract deposition. If the deposition was within the trachea or principal bronchi, then respiratory tract deposition is likely to be higher for both devices and gastrointestinal deposition likely to be lower for both devices. Areas for additional research include the following: assessing respiratory tract deposition in animals with respiratory disease; measuring breathing patterns and tidal volumes of healthy dogs and dogs with respiratory disease and correlating these with respiratory tract deposition; correlating respiratory tract deposition with clinical response to treatment in animals with respiratory disease; assessing the relationship between dosage of aerosolized medication with clinical response; analyzing the relationship between PSD and respiratory tract deposition in animals; investigating the relationship between the seal of face masks and decrease in respiratory tract deposition in animals; using 3D gamma scintigraphy to investigate peripheral versus central respiratory tract deposition in animals; and measuring exhaled or leaked aerosol during administration in animals to improve understanding of the generally low level of respiratory tract deposition achieved.

Conclusion

In conclusion, our study confirms respiratory tract deposition of aerosolized radiolabeled fluticasone propionate delivered from a nebulizer and MDI in healthy, tidally breathing dogs with and without sedation. Our results suggest that nebulization achieves more reliable respiratory tract deposition than does MDI treatment. Respiratory tract deposition in our dogs was low compared to reported deposition in adult humans but is similar to reported deposition in children <4–5 years old.

Footnotes

Acknowledgments

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