

Predicted Tracheobronchial and Pulmonary Deposition in a Murine Asthma Model

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ABSTRACT

Particulate matter dosimetry provides the critical link between exposures and initial doses reaching various sites in the respiratory tract. To extrapolate findings from animal models to humans, quantitative respiratory-tract anatomical data dosimetry in these animal models is required. The goal of this study was to provide anatomical information for the tracheobronchial and pulmonary region so predictions of particle deposition could be performed for a widely used model of asthma; the sensitized Balb/c mouse. Tracheobronchial airway morphometry of sensitized male Balb/c mice was generated from three in situ prepared lung casts. Distribution of the number of generations to terminal bronchiole for each lung lobe was determined by assigning a unique binary number to each airway. This strategy enabled the median path length to terminal bronchiole to be determined. A total of 25 median length paths to terminal bronchiole were measured (airway length, diameter, and branch angle) in each lung cast. These 25 paths were proportionately distributed among the six lobes based upon the number of median length pathways in each cast. Airway length, diameter, and branch angle were measured for each airway in the 25 median length pathways. Measurements of airway length, diameter, and branch angle for each generation were averaged to create a typical path tracheobronchial anatomy model. A pulmonary airway model was also developed so that particle deposition predictions could be performed for particle diameters of 0.2–10 micrometers. Particle deposition efficiency predictions were consistent with in vivo measured deposition. Anat Rec, 290:1309–1314, 2007. © 2007 Wiley-Liss, Inc.

Key words: tracheobronchial morphometry; airway deposition; Balb/c mice; strain differences

Use of murine models of asthma have led to several new insights in the pathophysiology of this common childhood syndrome (Mauderly, 2000; Lippmann et al., 2003; Wages et al., 2004; Taube et al., 2004; Pauluhn and Mohr, 2005). This includes the potential effect of inhaled ambient particulate matter in these asthmatic mouse models (Kleinman et al., 2005; Hao et al., 2003). In these studies, it is important to differentiate between an exposure level and the actual dose that elicits the measured response. Inhaled particulate matter dosimetry provides this critical link between exposures and the initial doses reaching various sites in the respiratory

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tract (NRC, 2004). A priori use of dosimetry models would enable dose predictions to various regions of the respiratory tract yielding improved study design and better extrapolation to humans.

Current inhaled particle dosimetry efforts are predominantly based upon the aerosol properties, anatomic structure, and physiological functioning of the respiratory tract, such as have been represented in various mathematical models (ICRP, 1994; NCRP, 1997; MPPD, 2002). These models have been verified for idealized particles (spherical, nonhygroscopic). The primary limitation in use of these dosimetry programs is a lack of quantitative respiratory tract morphometry. Recently, differences in airway morphometry in the first six tracheobronchial generations in two varieties of mice have been used to demonstrate that differences in their drug response ascribed to differences in drug metabolism are likely due to delivered dose (Moss and Oldham, 2006). The purpose of this study was to provide dosimetry predictions based upon quantitative tracheobronchial and pulmonary airway morphometry in a murine model of asthma.

MATERIALS AND METHODS

Animals

Details regarding the ovalbumin sensitized Balb/c mice used in this study and the methods used for preparing the *in situ* lung casts are described in previous morphometric work on the first six tracheobronchial generations (Phalen et al., 1973; Oldham and Phalen, 2002). All procedures involving mice were reviewed and approved by the Institutional Animal Care and Use Committee at the University of California, Irvine. Animals were housed in isolator cages on a 12-hr light/dark cycle and provided with food and water *ad libitum*. In preparation for lung casting mice were killed intraperitoneally with a lethal dose of sodium pentobarbital (120 mg/kg). Measurements of body characteristics (weight, length, chest circumference) were performed, and the upper trachea was cannulated through a slit just below the larynx. The chest was opened, and the lungs gently ($< 25^\circ \text{H}_2\text{O}$) filled and emptied with carbon dioxide from a compressed gas cylinder. After the last deflation, the lungs were filled with physiological saline that had been previously degassed under vacuum. Silicone rubber room-temperature vulcanizing (Silastic E, Dow, Midland, MI) was mixed, degassed, and then injected (0.15–0.2 ml/min) into the lungs displacing the saline (saline diffuses out the pleura) using a disposable plastic syringe and syringe pump. Based upon previous work a target volume in cc of 0.35% of body mass (in grams) was injected into each mouse lung. After injection, the cannula was removed, the mouse was covered with a plastic sheet, and the silicone rubber was allowed to cure for 24–30 hr. After curing, the lungs were removed and the tissue was dissolved in $\sim 6 \text{ M NaOH}$ solution. Subsequently, the NaOH was neutralized with vinegar; the lung cast was washed and stored in isopropyl alcohol. The accuracy of the *in situ* casting method has been demonstrated in rats using tantalum bronchograms (Yeh et al., 1975).

Tracheobronchial and Pulmonary Morphometry

Three of the six lung casts previously selected for quantitative morphometry of the first six tracheobron-

chial generations (Oldham and Phalen, 2002) were used in this study. For each of the three lung casts, the number of generations (trachea = generation 1) to every terminal bronchiole were counted. Casts were examined using a $10\times$ magnifying lens, which allowed terminal bronchioles to be recognized as the last nonalveolarized structure in a pathway. By assigning a unique binary number to each airway (Phalen et al., 1978) it was possible to track which airways had been counted and how many generations there were from trachea to each terminal bronchiole. Correction for any missing airways (which were few, but apparent on the lung casts) was done by counting a substitute pathway with an identical identification number from another cast. Based upon these data, the median number of generations from the trachea to terminal bronchioles was determined for each cast using a cumulative distribution plot. Twenty-five of these median length pathways were selected on each lung cast for measurement of airway length, diameter, and branch angle. Pathways from each of the six lobes of each lung were included in proportion to the lobar distribution of median paths by selecting every i^{th} pathway, where $25i$ was approximately equal to the total number of median length pathways. Measurements of airway length, diameter, and branch angle were performed using a hand-held $\times 10$ magnifier with a reticle. Resolution of the measurement scales on the reticle was 0.05 mm and 1 degree.

Measurements were sorted by generation and averaged together to form a typical path model. In this case, the term “typical” means that the pathway is an estimate of the average of all pathways in three casts that have the median number of generations. Consistent with previous tracheobronchial airway morphometry studies (Yeh and Schum, 1980; Oldham et al., 1994), it became clear that the number of airways did not increase as a power of 2 beyond the sixth generation because terminal bronchioles were present at generation 6 in some pathways, therefore, a method of estimating the number of airways at each generation had to be selected. On a semilogarithmic plot, a straight line was drawn between the number of airways in generation 6 (32 airways) and the mean number of terminal bronchioles at generation 15 (1582, mean number of terminal bronchioles counted on the three lung casts). The number of airways in each generation between 6 and 15 was determined from this line.

Definitive pulmonary morphometry measurements were not possible using the Balb/c mouse lung casts that were produced for tracheobronchial morphometry measurements. Therefore, the method of Yeh et al. (1979), for constructing the alveolar region of the rat by interpolation between terminal bronchioles (of known number) and alveoli of known size and number, was used to construct the pulmonary region for the Balb/c mouse. This method depends on several assumptions: (1) a pulmonary airway tube contains 20 alveoli; (2) regular dichotomous branching exists beyond the terminal bronchioles; (3) a single generation of alveolar sacs exists beyond several generations of respiratory bronchioles, if any, and alveolar ducts; (4) the same number of generations of respiratory bronchioles and alveolar ducts are found in each lobe, as well as, the entire lung; (5) the length to diameter ratio of the alveolar sac is similar to that in humans (Weibel, 1963); (6) either the number or diame-

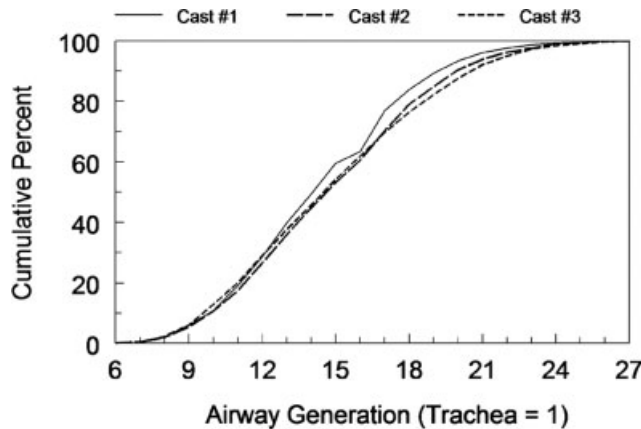


Fig. 1. Cumulative normalized distribution of number of generations to terminal bronchiole for three in situ replica lung casts of Balb/c mouse tracheobronchial tree.

ter of the alveoli is known; and (7) the total volume of the alveoli is approximately equal to 65% of the total lung capacity at full inspiration. Assumptions a and g are based upon the data of Weibel (1963) and are consistent with of the rat model of Granito (1971). Assumption b is based upon observations beyond the terminal bronchioles in the Balb/c mouse casts and is consistent with the observations of Yeh et al. (1979) for the Long-Evans black and white hooded rat. Assumption c is derived from assumption b and assumptions d–f were made for modeling simplicity.

In this pulmonary model, the total alveolar volume for a male 68-day-old Balb/c mouse reported by Kawakami et al. (1984), the mouse alveolar diameter of Mercer et al. (1994) and the equation of Taulbee and Yu (1975) were used to determine the total number of alveoli.

Deposition Efficiency Predictions

The typical path tracheobronchial and pulmonary dimensions along with a range of ventilation values were used as input into the NCRP (1997) dosimetry model to calculate predicted deposition efficiency for the tracheobronchial and pulmonary airways. The range of minute ventilation values were based upon experimental values measured by DeLorme and Moss (2002) for single (66 cc/min) and double plethysmograph (125 cc/min) in 20-g Balb/c mice and the predictions of Weibel (1972). Because predictive models for deposition efficiency in the nasal, oral, pharyngeal, and larynx (NOPL) regions have not been developed, predicted deposition in these regions using the approach of Oldham et al. (1994) were performed. This approach used the experimental data for the CF1 mouse of Raabe et al. (1988). Predicted deposition efficiency was calculated as a percentage of the mass of unit density spheres entering the nose.

RESULTS

The mean characteristics ($\pm$ SD) of the three 117-day-old male Balb/c mice whose lung casts were used in this study were as follows: body mass, 26.6 ± 0.03 g; rump to snout length, 10.2 ± 0.01 cm; chest circumference at

TABLE 1. Mouse lung cast characteristics

Cast no.	Range of generations in TB ^a	Median generation	No. of median generation paths	No. of terminal airways
1	7–27	15	140	1574
2	6–27	15	131	1612
3	7–27	15	134	1559

^aTB, terminal bronchioles.

axilla, 6.9 ± 0.1 cm; and chest circumference at xyphoid, 7.7 ± 0.5 cm.

Tracheobronchial and Pulmonary Morphometry

A total of 9,452 unique airways were counted and a total of 4,745 terminal bronchioles were identified on the three Balb/c mouse lung casts. The distribution of terminal bronchioles by generation was consistent among the three casts (Fig. 1), although there were slight differences (Table 1). The University of California, Irvine, typical path tracheobronchial airway model for the Balb/c mouse (Table 2) was used as input into the NCRP dosimetry code to predict particle deposition. As previously described, numbers of airways were determined by interpolation between generations 6 and 15. Using the method of Yeh et al. (1979) a model of the pulmonary airways of the male Balb/c mouse were developed (Table 3).

Deposition Predictions

Deposition in the extrathoracic airways removes most of the particles greater than 1.5 micrometers in diameter. The resulting inspiratory tracheobronchial and pulmonary deposition efficiency as a function of particle size for two ventilation levels that bracket the values measured by DeLorme and Moss (2002) for Balb/c mice are shown in Figure 2. Predicted tracheobronchial and pulmonary deposition results have been confined to the particle size range consistent with the NOPL deposition data of the Raabe et al. (1988).

DISCUSSION

The consistency of the number of total airways and airway measurements (measured by the standard deviation) between the three in situ Balb/c lung casts were consistent with previous work for the B6C3F₁ mouse (Oldham et al., 1994). As expected for submicron diameter particles, predicted inspiratory pulmonary deposition efficiency is substantially larger than predicted inspiratory tracheobronchial deposition efficiency (Fig. 2). Because deposition in the extrathoracic airways removes most of the particles greater than 1.5 micrometers in diameter, predicted inspiratory tracheobronchial and pulmonary deposition efficiencies are low for these particle diameters.

The differences in airway diameter in the first six tracheobronchial airway generations previously reported (Oldham and Phalen, 2002) between the Balb/c and B6C3F₁ mouse perpetuate down the tracheobronchial tree. These differences result in a greater discrepancy in predicted inspiratory tracheobronchial deposition effi-

TABLE 2. Typical path tracheobronchial geometry for the Balb/c mouse (means and SE)

Airway generation no. (trachea = 1)	Airway length (mm)		Airway diameter (mm)		Branch angle (degrees)		Gravity angle ^a (degrees)		No. of airways
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
1	10.2 ¹	0.6 ¹	1.10	0.04	0	0	90	0	1
2	4.6	0.41	1.03	0.05	14	1	105	3	2
3	1.91	0.19	0.95	0.09	25	6	98	4	4
4	0.69	0.14	0.72	0.07	26	6	91	1	8
5	0.96	0.08	0.64	0.04	18	3	78	3	16
6	0.58	0.06	0.52	0.04	18	2	76	3	32
7	0.54	0.06	0.48	0.03	12	3	60	N/A	49
8	0.47	0.03	0.40	0.03	22	4	60	N/A	76
9	0.39	0.03	0.36	0.02	16	3	60	N/A	117
10	0.36	0.03	0.35	0.02	9	2	60	N/A	181
11	0.33	0.02	0.32	0.04	24	4	60	N/A	279
12	0.28	0.02	0.24	0.01	20	3	60	N/A	431
13	0.25	0.02	0.21	0.01	16	3	60	N/A	664
14	0.21	0.01	0.16	0.01	26	3	60	N/A	1,026
15	0.16	0.01	0.10	0.00	56	3	60	N/A	1,582

^aTaken from previous morphometric data published by Oldham and Phalen (2002) on six in situ lung casts of Balb/c mice, including the three used in this work. Gravity angle for the trachea was assumed to be 90°. Generations 2–6 used the trachea as a reference, and generations 7–15 were assigned a gravity angle of 60°, as unbiased direct measurements were not possible.

TABLE 3. Typical path pulmonary model for the Balb/c mouse by the method of Yeh et al. (1979)

Airway generation no. (trachea = 1)	Airway length (mm)	Airway diameter (mm)	Branch angle (degrees)	Gravity angle ^a (degrees)	No. of airways
16	0.15	0.099	45	60	3,164
17	0.15	0.098	45	60	6,328
18	0.14	0.097	45	60	12,656
19	0.14	0.096	45	60	25,312
20	0.13	0.095	45	60	50,624
21	0.12	0.094	45	60	101,248
22	0.11	0.092	45	60	202,496
23	0.048	0.058	45	60	6.65 × 10 ⁶

^aTaken from previous morphometric data published by Oldham and Phalen (2002) on six in situ lung casts of Balb/c mice, including the three used in this work. Gravity angle for the trachea was assumed to be 90°. Generations 2–6 used the trachea as a reference, and generations 7–15 were assigned a gravity angle of 60°, as unbiased direct measurements were not possible.

ciency (calculated as percentage of concentration entering the nares) than previous reported (Fig. 3). This comparison highlights one of the limitations of this study, which is the assumption of equivalent deposition efficiency in the NOPL region for these two varieties of mice. Potential differences in NOPL deposition between these two varieties of mice could ameliorate or exacerbate the discrepancies shown in Figure 3. The same conclusion is true for deposition in the pulmonary region. Not only is there a lack of strain-specific alveolar diameters, but also strain-specific morphological shape data are not available. For example, Tenney and Reemers (1963) reported a 46.6- μ m mouse alveolar diameter compared with the 58- μ m mouse alveolar diameter reported by Mercer et al. (1994) that was used in this study. Although the strain of mouse used was not specified by either author, we chose to use the Mercer et al. (1994) data due to the rigorous morphological techniques used in this study. Recently, differences in alveolar size in inbred mouse strains (C3H/HeJ, C57BL/6J, and A/J) have been quantified (Soutiere et al., 2004); however,

because no comparison of alveolar shape was made, it is not possible to determine the effect on predicted deposition.

There is limited experimental data to compare with these deposition predictions. Most experimental studies do not measure ventilation parameters (breathing frequency and tidal volume) and do not measure particles that are cleared from the respiratory tract. Raabe et al. (1988) showed that, for exposures as short as 15 min, significant amounts of deposited material cleared into the gastrointestinal system. For some particle sizes, this amounted to over 60% of the deposited material. Wong et al. (2003) used exposure durations ranging from 7–15 min in Balb/c mice and found significant amounts of deposited material was cleared to the gastrointestinal tract. The data of Raabe et al. (1988) for tracheobronchial and pulmonary deposition data for the CF1 mouse was used to compare with predicted inspiratory and expiratory tracheobronchial and pulmonary deposition for the Balb/c mouse for two levels of ventilation (Fig. 4). The experimental data of Raabe et al. (1988) in the CF₁

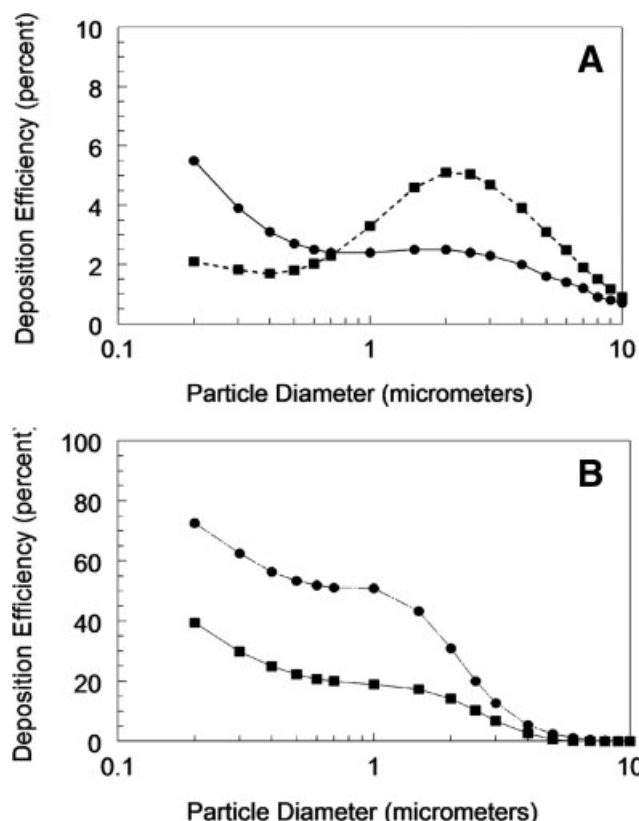


Fig. 2. Predicted inspiratory tracheobronchial (A) and pulmonary (B) particle deposition for minute ventilations of 25 (circles) and 125 (squares) ml in the Balb/c mouse lung model.

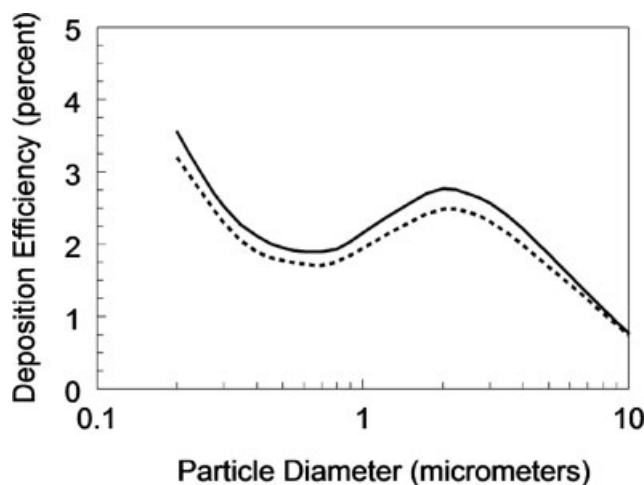


Fig. 3. Comparison of predicted inspiratory tracheobronchial deposition efficiency between the Balb/c mouse (solid line) and the B6c3F1 mouse (dashed line).

mouse were corrected to include some of the radioactivity found in the gastrointestinal region due to particle clearance (Oldham et al., 1994). The original reported aerodynamic resistance diameter (Raabe et al., 1988) was converted to aerodynamic equivalent diameter using

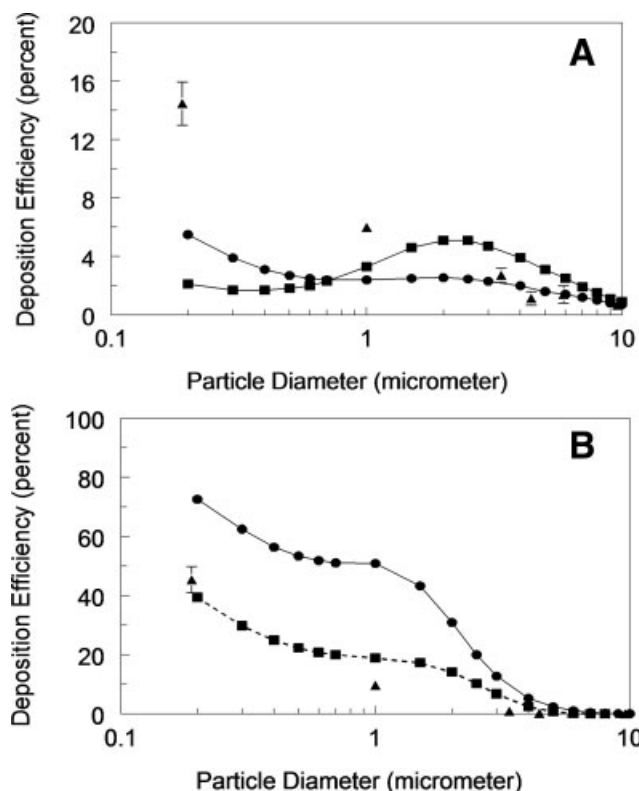


Fig. 4. Comparison of predicted inspiratory and expiratory deposition efficiency using the Balb/c mouse lung model for tracheobronchial (A) and pulmonary (B) regions for minute ventilations of 25 (circles) and 125 (squares) ml and the CF1 in vivo data (triangles) of Raabe et al. (1988). Error bars are standard deviations, and in some cases, were smaller than the symbol used to plot the point.

the method of Raabe (1976). Deposition occurring during the pause in the respiratory breathing pattern was neglected in the predicted deposition because of the high breath frequency (332–572 breaths/min) reported for the Balb/c mouse by DeLorme and Moss (2002). The agreement with the experimental data of Raabe et al. (1988) is reasonable considering the potential difference between strains of mice (CF1 and Balb/c).

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