The Effect of Lung Structure on Mucociliary Clearance and Particle Retention in Human and Rat Lungs

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Mucociliary clearance velocities in human and rat bronchial airways were calculated in asymmetric, multiple-path models of the bronchial tree by solving mass transport equations based on the assumption of conservation of mucus volume and normalized to measured tracheal mucus velocities of 5.5 mm/min for humans and 1.9 mm/min for rats. Mucus velocities in single airways of the rat lung exhibited a significant statistical relationship with airway diameters but not with generation numbers, while both parameters provided equally suitable relationships for the human lung. Retention curves reflecting the combined effects of deposition and clearance were computed for unit density particles of 0.1, 1, 2, and 7 μm for resting breathing conditions. About 10 to 15% of the particles initially deposited in the human bronchial tree were still retained after 24 h, while most of the particles deposited in the rat bronchial tree were cleared after about 6 to 8 h. Snapshots of the distributions of mass retained among human bronchial airways at different times after the end of exposure indicated that the observed slow bronchial clearance may partly be attributed to delayed mucociliary clearance from particles initially deposited in the most peripheral conductive airways. If plotted as functions of airway diameter, human and rat bronchial retention patterns exhibit very similar shapes in contrast to their dependence on airway generation number. Thus, extrapolation of toxicologic response, based on local retention patterns from rat to human exposures, should be based on airway diameter rather than on generation number.

Key Words: human lung; rat lung; mucociliary clearance; particle retention; extrapolation modeling.

Upon inhalation, particles initially deposited on airway surfaces are subsequently cleared by various clearance mechanisms. Depending on their initial site of deposition, a fast- and a slow-clearance phase have been observed in particle retention experiments. The fast-clearance phase has traditionally been interpreted as tracheobronchial (TB) clearance, whereas the slow-clearance phase is commonly attributed to mechanical clearance from the alveolar region (ICRP, 1994). While there is general agreement that mucociliary transport is the principal clearance mechanism in the TB region during the first 24 h of exposure, experimental data from bolus inhalation studies postulated the existence of a slow bronchial clearance phase (Stahlhofen et al., 1990). However, recent calculations of mucociliary clearance in the human TB tree suggested that these findings might be partly explained by a delayed clearance from peripheral bronchiolar airways in an asymmetric lung structure (Asgharian et al., 2001b).

For experimental reasons, direct measurements of mucus velocity in human lungs are presently available only for the trachea (Foster et al., 1982; Leikauf et al., 1981; Mussatto et al., 1988; Toomes et al., 1981; Yeates et al., 1975, 1981, 1982). The use of different experimental techniques produced wide variability in the measured values, ranging from 3.6 to 21.5 mm/min. Average tracheal mucus velocities in healthy nonsmokers, as measured by noninvasive radiological techniques, ranged from 4 to 6 mm/min; consequently, both ICRP (1994) and NCRP (1997) committees adopted a value of 5.5 mm/min, which was also used in the present study. The only reported value of the mucus velocity in the main bronchi is about 2.4 mm/min (Foster et al., 1980).

Tracheal mucociliary clearance velocities in rats display a wide range of values, presumably because of differences in measurement techniques. Values found in the literature are 8.1 mm/min (Giordano and Morrow, 1972), 5.1 mm/min (Patrick and Stirling, 1977), and 1.9 mm/min (Felicietti et al., 1981). Because the two earlier studies used anesthesia, which has been shown to influence mucociliary transport, the mean tracheal velocity of 1.9 mm/min determined by Felicietti et al. (1981) was used in the present calculations.

Because of the very limited experimental data on mucus clearance velocities in human bronchial airways, mucus velocity in other bronchial airways has to be calculated, using assumptions about airway morphology and properties of the mucus flow in these airways (ICRP, 1994; NCRP, 1997). Presently available data on mucus velocities in human TB airways are commonly based on deterministic, symmetrically branching lung structures (ICRP, 1994; NCRP, 1997). Recently, however, Asgharian et al. (2001a) demonstrated that the asymmetry of the branching pattern as well as the variability of linear airway dimensions must be considered for realistic calculations of mucus velocities in bronchial airways.
Calculations of bronchial mucus velocities in the rat lung are currently also based on symmetric, deterministic lung morphologies (Hofmann et al., 1993), thereby neglecting the asymmetry of the branching pattern, which is particularly noticeable in the rat lung. Preliminary results indicated that airway asymmetry might also play an important role in the determination of mucus velocities in the rat TB tree (Hofmann and Asgharian, 2002).

The objectives of the present study were threefold: (1) to calculate mucus velocities for an asymmetric, multiple-path model of the bronchial airways in the rat lung and to compare the results to the corresponding data for the human lung (Asgharian et al., 2001b); (2) to relate the variability of mucus clearance velocities to the heterogeneity of the corresponding airway structure in both human and rat TB trees and to identify interspecies differences; and (3) to predict retention patterns for different particle sizes as a result of the combined effect of deposition and clearance in human and rat lungs, which might then serve as a basis for interspecies extrapolation studies.

**MATERIALS AND METHODS**

Particle deposition and clearance in bronchial airway generations were calculated in asymmetric, multiple-path models of the bronchial tree of the human and rat lungs.

Multiple-path models are asymmetric, deterministic models—i.e., each airway from the trachea to the terminal bronchioles has defined linear dimensions and a defined sequence of airways corresponding to the branching asymmetry of a specific lung.

In the case of the rat lung, Raabe et al. (1976) provided a complete morphometric description of the TB tree of a Long-Evans rat. Hence these data could directly be used to construct an asymmetric multiple-path model of the rat lung (Anjilvel and Asgharian, 1995). In the case of the human lung, Raabe et al. (1976) measured practically all bronchial airways, while only a few selected paths were traced down to the level of terminal bronchioles. This limited database was then used by Koblinger and Hofmann (1985) to develop a complete asymmetric stochastic model of the human bronchial tree. Based on this stochastic model, 10 asymmetric, structurally different, multiple-path models of the TB tree were constructed that varied in linear airway dimensions, number of bronchial airway generations, and, consequently, number of terminal bronchioles (Asgharian et al., 2001a; Hofmann et al., 2002). Thus, all subsequent results of deposition and mucociliary clearance calculations represent averages of 10 structurally different bronchial trees.

The approach for computing particle transport and deposition in a multiple-path model has been described in detail by Anjilvel and Asgharian (1995). In essence, splitting of airflow, and hence particle concentration, at bronchial airway bifurcations is related to distal volumes. Deposition efficiency equations for particle deposition in cylindrical bronchial airways were those proposed by Ingham (1975) for diffusion, Cai and Yu (1988) for impaction, and Wang (1975) for sedimentation. Respiratory parameters refer to human resting breathing conditions, (tidal volume, TV = 750 ml and breathing frequency, f = 12/min) (ICRP, 1994), and quiet breathing conditions for the Long-Evans rat (TV = 2.1 ml and f = 102/min) (Anjilvel and Asgharian, 1995). Particle deposition fractions were then computed in all bronchial airways of the human and rat TB geometries. Since we wanted to investigate potential differences in the distribution of retained particles among bronchial airways, deposition fractions were normalized to the number of particles entering the trachea, i.e., ignoring deposition in extrathoracic airways.

Four assumptions regarding mucus flow in the conductive airways are required to calculate mucus velocities (Asgharian et al., 2001b): (1) the mucus layer has an effective thickness that is constant locally for a single bifurcation; (2) the net effective velocity, considering discontinuities of the mucus layer and mucus production, is constant in any given airway; (3) mucus production rates are the same in all terminal bronchioles; and (4) the mucus layer thickness is small compared to the airway diameter.

Mucus clearance velocities in each airway were calculated for steady-state, steady-flow conditions based on the assumption of conservation of mucus volume (Asgharian et al., 2001b). Expressing mucus volume flow rate as mucus volume in an airway divided by the mucus residence time in that airway and expressing the residence time in terms of airway length and mucus velocity, conservation of mucus volume in an airway is given by

\[
VD = V_d D_d + V_e D_e \quad (1)
\]

where \(V\) and \(D\) are the net mucus velocity and airway diameter, and subscripts \(d1\) and \(d2\) refer to the two daughter branches of that airway. Based on mucus volume balance between the trachea (generation 1) and the terminal bronchioles (last bronchial airway generation), calculations of mucus velocities start at the terminal bronchioles. By traversing up the bronchial tree, mucus velocities were calculated for each bronchial airway. Mucus velocities in all bronchial airways refer to measured tracheal mucus velocities of 5.5 mm/min for humans (ICRP, 1994; NCRP, 1997) and 1.9 mm/min for rats (Felicietti et al., 1981).

Transport of particles initially deposited in the alveolar region to the TB region by alveolar macrophages was not considered here because we wanted to eliminate any contribution from alveolar clearance.

Finally, mass retained at time \(t\) in bronchial airways was found by adding the calculated retained mass in each airway of the conducting tree at time \(t\) to obtain the overall mass in the TB region. For a given airway, the particle mass balance yields

\[
dm/dt = \dot{m}_{ret} + \dot{m}_{d1} + \dot{m}_{d2} \quad (2)
\]

where \(m\) is the mass retained in the airway, \(\dot{m}_{ret}\) is the deposition rate, \(\dot{m}_{d1}\) and \(\dot{m}_{d2}\) are the mass rates entering the airway from the two daughter branches, and \(\dot{m}_{ret}\) is the mass rate leaving that airway. To find the retained mass in each airway, the set of particle mass balance equations was solved at consecutive small time increments from the start of the exposure to the end of the post exposure period (Asgharian et al., 2001b). Traversing up the bronchial tree, the mass retained in each airway is calculated until the trachea is reached. Once the retained mass in all airways is found, they are added to determine the mass retained in the TB region at the end of each time increment. Computations are carried out from the start of exposure to the end of the post-exposure period or until the mass in the TB region becomes negligible. The exposure scenario adopted in the present study assumes 1 h of exposure followed by 48 h of post exposure.

**RESULTS**

Mucus velocities in each bronchial airway of the human and rat lungs are plotted in Figures 1 and 2 as functions of airway generation numbers. In each airway generation, mucus velocities display a wide range of values. This variability illustrates the asymmetric and random branching pattern of the human and rat airway system, as clearance velocities would be identical in all airways of a given airway generation in the case of a symmetric, deterministic TB tree (ICRP, 1994; NCRP, 1997). Because of mass balance considerations, the clearance velocity in a given airway depends not only on the diameter and length of that airway, but also on the mucus volume in distal daughter airways entering the corresponding parent airway.

Inspection of Figure 1 suggests that a statistical relationship exists between mucus velocity and airway generation number. Indeed, average mucus velocities in the human lung decrease in a roughly exponential fashion with increasing airway gen-
eration number (Hofmann and Asgharian, 2002). Due to the asymmetric branching scheme of the human lung, the total number of bronchial airways along different paths can vary considerably. As a result, airways in a given generation of the peripheral region of the lung can still be conductive or already alveolated (i.e., a bronchial airway may still be found in generation 21 [trachea = generation 1], although with a very small probability).

In the rat lung, however, the data plotted in Figure 2 show a much weaker correlation, which is an indication of the monopodial structure of the rat TB tree as compared with the more symmetric branching pattern in the human lung. In an earlier study, average mucus velocities displayed a distinct two-exponential relationship in the rat lung as a result of its asymmetric branching structure, although a significant variability of mucus velocities in different airways of the same generation could be observed (Hofmann and Asgharian, 2002). Because of the significantly more asymmetric branching of the rat lung as compared with the human lung, bronchiolar airways can extend even further down to generation 33.

Previous calculations of particle deposition patterns have indicated that airway diameter is a more appropriate parameter for characterizing local particle deposition patterns in the rat lung than the commonly used airway generation numbers (Hofmann et al., 1999), while both are equally suitable for the human lung. Hence, the question arises whether this also applies to local mucociliary clearance patterns.

Mucus velocities in each bronchial airway of the human and rat lung are plotted in Figures 3 and 4 as functions of airway diameter. The variability and asymmetry of linear airway dimensions and the history of downstream mucus transport again cause the observable scatter of the data. The reduction of the scatter of the velocities is particularly noticeable for the rat lung when compared with the airway generation plot (Fig. 2).

FIG. 1. Distribution of mucus velocities among human bronchial airway generations based on 10 stochastically generated, multiple-path models of the human bronchial tree.

FIG. 2. Distribution of mucus velocities among rat bronchial airway generations based on a multiple-path model of the rat bronchial tree.

FIG. 3. Relationship between mucus velocities in human bronchial airways as a function of airway diameter, based on 10 stochastically generated, multiple-path models of the human bronchial tree.
Here, mucociliary clearance velocities in single airways exhibit a significant statistical relationship with airway diameters, illustrating again the monopodial nature of the branching pattern in the rat lung. The observed correlation between mucus velocity and airway diameter is most likely due to the fact that mucus velocity is diameter-dependent in the mass balance equations (Asgharian et al., 2001a). This correlation suggests that airway diameter is indeed a more appropriate morphometric parameter for local mucus clearance patterns in an asymmetric lung than airway generation number. The best fit to the data for rats was obtained by the relation

\[ m_v = 1.9(1 - \exp(-7.0743 d^{1.7971})) \quad (R^2 = 0.80), \quad (3) \]

where \( m_v \) is the mucus velocity (mm/min) and \( d \) is the airway diameter (cm). The distinct asymmetry of main and lobar bronchial diameters, and hence, related mucus velocities, does not permit a better mathematical fit for the large bronchial airways.

In case of the human TB tree, both airway generation number and airway diameter provide comparable fits. For example, the relationship between mucus velocity and airway diameter is given by the equation

\[ m_v = 5.5(1 - \exp(-0.49621 d^{2.2604})) \quad (R^2 = 0.95). \quad (4) \]

Finally, particle retention, which reflects the combined effects of deposition and clearance, was computed for unit density particles of 0.1, 1, 2, and 7 \( \mu \)m for a scenario of 1 h of exposure followed by 48 h of post exposure. Bronchial retention curves (i.e., the fraction of mass retained in the TB region as a function of time) for both human and rat lungs are displayed in Figures 5 and 6, respectively. Depending on particle diameter, half-times of mucociliary clearance, \( T_{0.5} \), range from approximately 4 to 9 h, with a weighted average value of about 8 h. After 24 h, 10 to 15% of the particles initially deposited in the bronchial tree were still retained in the human lung (Fig. 5), suggesting that long-term retention may be partly attributed to delayed bronchial clearance. The observed relationships between clearance half-time and slow-clearance fraction on particle diameter are caused in this model by differences in their initial deposition patterns, since mucociliary clearance velocities per se do not depend on particle size.

An important feature of the experimental observations of Stahlhofen et al. (1990) is that the slow-bronchial-clearance fraction in the human lung decreases with rising geometric particle diameter, eventually disappearing for particle sizes larger than about 6.7 \( \mu \)m. These experimental findings are not supported by the present simulations. Thus, additional slow-clearance mechanisms must be operating in human bronchial airways for particles smaller than 6.7 \( \mu \)m in diameter, which are eventually responsible for the experimentally observed size dependence (ICRP, 1994). However, that size dependence reported for humans could be observed in the rat, suggesting that the inhomogeneity of mucociliary clearance in the rat is indeed the primary slow-clearance mechanism.

Clearance of particles deposited initially in the bronchial airways is much faster in the rat than in the human lung. Half-times of mucociliary clearance, \( T_{0.5} \), range from about 1 to

FIG. 4. Relationship between mucus velocities in rat bronchial airways as a function of airway diameter based on a multiple-path model of the rat bronchial tree.

![Graph showing mucus velocity vs. airway diameter](image)

**FIG. 5.** Fraction of mass retained in the bronchial region of the human lung after 1 h of exposure to unit density particles of 0.1, 1, 2, and 7 \( \mu \)m.
1.5 h, with an average value of approximately 1.25 h, and most particles were cleared after about 6 h (Fig. 6). However, a small fraction still remains in the bronchial tree even after 24 h, except for the 7-μm particles, suggesting the existence of a slow bronchial phase in the rat TB tree. The observed dependence of clearance half-time and slow-clearance fraction on particle diameter in the rat TB tree is again caused by differences in their initial deposition patterns.

Distributions of retained mass among human and rat TB airway generations at different times after a 1-h exposure period were computed for unit density particles of 0.1, 1, 2, and 7 μm. Since all distributions displayed the same tendencies, only the results for particles of 0.1 (diffusion-dominated) and 2 μm (impaction dominated) are shown in Figures 7 and 8 for humans and rats, respectively. To facilitate comparison between human and rat retention patterns, mass distributions were computed for t = 0 (end of exposure period), t = T_{0.5} (clearance half-time, i.e., 50% is still retained) and t = T_{0.1} (i.e., 10% is still retained). The retention patterns at the end of exposure essentially represent the initial deposition patterns. With increasing time after exposure, retained mass decreases in the TB region. In the human lung, most of the mass initially deposited in the large bronchial airways is rapidly cleared in the first few h after cessation of inhalation. After about 8 h, the relative distribution hardly changes any more, illustrating the existence of steady-state conditions. In the rat TB tree, however, mass is continuously transported toward the trachea, thereby shifting the retention patterns to proximal airway generations. This continuous transport suggests that mass is cleared in each generation by approximately the same rate at all times, at least within the first 4 h after the end of exposure.

Comparison of the retention patterns, shown in Figures 7 and 8 as a function of airway generation number, reveals that the TB retention distributions are distinctly different in humans as compared with rats. While the retained mass in the human lung exhibits a peak in distal bronchiolar airways, retention in the rat lung has a broad maximum in upper bronchial airways. (Note: The minimum of retained mass in generation 4 of the
human TB tree is caused by the minimum airway volume in that generation [Raabe et al., 1976]). Hence, both retention patterns may produce different toxicologic responses. However, this difference could also be interpreted to indicate that airway generation number is simply not the most appropriate morphometric parameter for extrapolation purposes, as has already been demonstrated for particle deposition patterns (Hofmann et al., 1999).

Thus the retention patterns for both human and rat lungs at different times after the end of a 1-h exposure, as shown above in Figures 7 and 8, were replotted in Figures 9 and 10 as average retention per bronchial airway diameter as a function of that airway diameter. (Note: the minima of retained mass in the human TB tree correspond to the minimum in airway generation 4 in Fig. 7). When comparing human and rat reten-

**FIG. 8.** Mass retained in the rat TB region at different times after the end of a 1-h exposure to unit density particles of 0.1 (A) and 2 μm (B), plotted as a function of bronchial airway generation numbers.

**FIG. 9.** Average mass retained in the human TB region at different times after the end of a 1-h exposure to unit density particles of 0.1 (A) and 2 μm (B), plotted as a function of bronchial airway diameters.
tion patterns, two distinct features can be observed. (1) The shapes of the retention curves in the human TB tree are practically identical at all times for airway diameters below about 0.3 cm and also between \( t/T_{0.5} \) and \( t/T_{0.1} \) for the large bronchial airways. The significant drop of the retained mass in the large bronchi between end of exposure and clearance half-time illustrates the preferential rapid removal of particles in these airways shortly after the end of exposure. In the rat lung, however, all three retention curves are very similar, indicating that relative clearance rates were similar in all bronchial airways at all times (2). In contrast to the airway generation plots (Figs. 7 and 8), both human and rat retention curves increase with rising diameter in a very similar fashion. This apparent similarity in retention curves suggests that airway diameters are a more appropriate morphometric parameter for the comparison of particle retention patterns in human and rat lungs than the commonly used airway generations.

**DISCUSSION**

In the case of the relatively symmetric human lung, either airway generations or airway diameters have been proposed to characterize the local morphology of bronchial airways (Phillips et al., 1994; Raabe et al., 1976). Because of the strong statistical correlation between generation numbers and airway diameters, both parameters seem to be equally suitable for the human lung (Koblinger and Hofmann, 1985). In the case of the monopodial structure of the rat lung, however, airway diameter is a much better structural parameter than generation number (Koblinger and Hofmann, 1988; Phillips and Kaye, 1995).

In the present study, mucociliary clearance velocities in bronchial airway generations were calculated in asymmetric, multiple-path models of the bronchial tree of the human and rat lungs. Computed average mucus velocities in human bronchial airways could be expressed mathematically, either as functions of airway diameter or of generation number. In the rat lung, however, average mucus velocities in bronchial airways exhibited a significant statistical relationship with airway diameters but not with generation numbers. This illustrates that mucus velocities are closely correlated with the structure of the bronchial tree.

Previous calculations of particle deposition patterns have indicated that airway diameter is a more appropriate parameter for characterizing local particle deposition patterns in the rat lung than airway generation number (Hofmann et al., 1998), while both are equally suitable for the human lung. Our present simulations indicate that this is also true for local retention patterns. If plotted as functions of airway generation number, distributions of retained mass among bronchial airways are distinctly different between the human and rat TB trees. If plotted as functions of airway diameter, however, human and rat bronchial retention patterns exhibit remarkably similar shapes. Since toxicologic response may be closely related to local retention patterns, extrapolation of local toxicologic response from rat to human exposures should be based on airway diameter rather than on the commonly used airway generation numbers.

Retention curves (Fig. 5), reflecting the combined effects of deposition and clearance, indicated that about 10 to 15% of the particles initially deposited in the human bronchial tree were still present after 24 h. Snapshots of the distributions of mass retained among bronchial airways, at different times after the...
end of exposure (Fig. 7), indicate that the observed slow bronchial clearance may be partly attributed to delayed mucociliary clearance from particles initially deposited in the most peripheral conductive airways. Previous simulations (Asgharian et al., 2001b) have demonstrated that the fraction retained after 24 h exhibits significant intersubject variations—i.e., 24-h values are higher in larger lungs than in smaller lungs if based on the same tracheal mucus velocity.

The dependence of the slow-clearance fraction on particle diameter observed in our calculations is caused entirely by differences in initial deposition patterns. The experimental findings of Stahlhofen et al. (1990) that the slow bronchial clearance fraction decreases with rising geometric particle diameter in the human lung were not borne out by our simulations. This suggests that additional slow-clearance mechanisms may be operating in human bronchial airways, which are eventually responsible for the experimentally observed size dependence (ICRP, 1994), such as the penetration through the mucus gel layer and subsequent removal by airway macrophages at a much slower rate. For example, Gehr et al. (1991) proposed that particles deposited on bronchial airway surfaces are immediately covered by the surfactant, which helps to overcome the surface tension so that smaller particles are dragged through the mucus with higher probability than larger particles. Alternatively, this size dependence may be caused by temporary local discontinuities of the mucus gel layer, where smaller particles have a higher chance to penetrate a patchy gel layer and to deposit on the sol layer than larger particles (Sturm and Hofmann, in press).

In contrast to the human lung, all particles in the rat bronchial tree were cleared after about 6 to 8 h. While slow bronchial clearance has so far been demonstrated only for humans, our calculations suggest that this should also be considered for the rat lung. However the size dependence of the slow-clearance fraction predicted for the rat is consistent with the human findings, suggesting that the inhomogeneity of mucociliary clearance in the rat is indeed the primary slow-clearance mechanism.

Local retention patterns plotted in Figures 7 through 10 refer to endotracheal inhalation conditions. The removal of particles by human and rat nasal passages upon inspiration can be characterized by the nasal penetration probability (Hofmann and Bergmann, 1998). Likewise, inhalability in rats drops already at sizes of about 1 μm, while it can be neglected in the human lung below about 10 μm (Ménache et al., 1995). Thus the fraction of inhaled particles of a given size actually retained in bronchial airways can be obtained by multiplying the computed retention fractions with the penetration probability and inhalability of that particle size. While the consideration of extrathoracic deposition and inhalability reduces the mass retained in the TB region, it does not modify the relative distributions of particles retained among bronchial airways for a given particle diameter.

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