Effect of aging on tracheal mucociliary clearance in beagle dogs

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WHALEY, SANDRA L., BRUCE A. MUGGENBURG, FRITZ A. SEILER, AND RONALD K. WOLFF. Effect of aging on tracheal mucociliary clearance in beagle dogs. J. Appl. Physiol. 62(3): 1331-1334, 1987.—Tracheal mucous velocity measurements were made in 24 beagle dogs in five age groups, using a gamma camera to detect movement of instilled $^{99m}$Tc-macroaggregated albumin. Age groups were defined as immature (9-10 mo), young adult (2.8-3.0 yr), middle aged (6.7-6.9 yr), mature (9.6-9.8 yr), and aged dogs (13.6-16.2 yr). Mean velocities were 3.6 ± 0.4 (SE) mm/min in the immature dogs, 9.7 ± 0.6 mm/min in the young adults, 6.9 ± 0.5 mm/min in the middle-aged dogs, 3.5 ± 0.8 mm/min in the mature dogs, and 2.9 ± 0.5 mm/min in the aged dogs. Tracheal mucous velocity was significantly (P < 0.05) greater in the young adult and middle-aged groups compared with the immature, mature, and aged dogs. This pattern of age-related changes was noted to be similar to age-related changes described for certain pulmonary function measurements.

Tracheal mucous velocity; age groups; age-related changes

TRACHEAL MUCOUS CLEARANCE is an important defense mechanism for the removal of deposited particles from the conducting airways of the lungs. Clearance rates of particles deposited in the trachea are affected by a variety of substances and insults, such as anesthetics (6, 9), other pharmaceuticals (2, 3), pollutants, and environmental factors (16). Biologic conditions, such as diseases (18), also influence tracheal mucous clearance rates.

The possibility that aging significantly affects mucociliary clearance and thus compromises the body's ability to clear the respiratory tract of potentially toxic material may have important implications. Age effects on other pulmonary functions and respiratory anatomy of dogs and humans have been reviewed by Mauderly (10) and Mauderly and Hahn (11). However, there is a paucity of studies relating age to mucociliary clearance. Goodman et al. (8), in examining tracheal mucous velocity of nonsmokers, young smokers, exsmokers, and patients with chronic bronchitis, found young (19-28 yr) nonsmokers had a tracheal mucous velocity (mean ± SE) of 10.1 ± 1.1 mm/min. Older (56-70 yr) nonsmokers had a significantly lower tracheal mucous velocity of 5.8 ± 0.98 mm/min. Puchelle et al. (13) showed that bronchial mucociliary clearance was inversely related to age in nonsmokers of three different age groups. No long-term longitudinal studies involving the same individuals have been done.

The goal of this study was to examine the effect of aging on tracheal mucociliary clearance rates in dogs. Beagle dogs were chosen as the experimental animal for several reasons. Felicetti et al. (5) have shown that canine tracheal mucociliary clearance patterns are very similar to those of humans. Tracheal velocities were measured so that clearance at a known site could be measured. We wished to avoid possible effects of altered deposition of inhaled marker particles that would in turn alter observed clearance rates. By use of a well-maintained dog colony (1), it was possible to measure tracheal mucous clearance in the same group of beagle dogs over years (3-10 yr of age) while they were maintained under similar well-defined environmental and nutritional conditions. This meant that many confounding variables could be controlled. It was possible to supplement these data with measurements of both young and old dogs from the same colony. The continuity of conditions in the colony and a well-defined breeding program (1) minimized possible differences between different measurement times and groups. Beagle dogs were also large enough to facilitate the use of a fiber-optic bronchoscope.

METHODS

Animals. Twenty-four beagle dogs from the Inhalation Toxicology Research Institute (ITRI) colony were used in this study. Longitudinal measurements were made in five males and three females when they were young adults (2.8-3.0 yr), middle-aged adults (6.7-6.9 yr), and mature adults (9.6-9.8 yr). Additionally, five females and three males, age 9-10 mo, comprised the immature dogs, and four males and four females, age 13-16 yr, were designated as the aged dogs. The animals were housed two or three dogs per run in kennels with indoor-outdoor runs, were fed dry kibble at 350 g/day (Wayne Dog Food, 1331...
Allied Mills, Chicago, IL), and were given water ad libitum. All dogs received physical examinations, thorax radiographs, complete blood counts, and serum chemical determinations of glutamic-pyruvic transaminase, alkaline phosphatase, blood urea nitrogen, and creatinine before entering this study. No evidence of systemic or respiratory disease was found in any of the dogs. Review of each dog’s history revealed no disease process that would have influenced tracheal mucous clearance. One aged animal (dog 6) was on thyroid hormone replacement therapy for confirmed hypothyroidism.

**Intratracheal instillation.** Food and water were withheld for at least 8 h before anesthesia was given. Anesthesia was induced using 5% halothane in O₂ given by face mask. When palpebral and pedal reflexes were gone, the dogs were intubated with an endotracheal tube. Dogs were then placed in a sling and positioned upright with their right sides parallel to the front of a gamma camera (Picker 4/15, Northford, CT).

The tracheal mucous clearance measurement procedure was a modification of the technique used by Chopra et al. (4) as described previously (19). A 4.8-mm-diam Olympus fiber-optic bronchoscope (model BF4B2, Olympus, Lake Success, NY) was passed into the trachea via the endotracheal tube. A 1.5-mm-OD polyethylene catheter was introduced through the biopsy channel of the bronchoscope and visually located at the instillation site. A 10-μl droplet of normal saline containing 20 μCi of ⁹⁹ᵐTc-macroaggregated albumin was deposited via a catheter onto the mucosa of the trachea near the bifurcation of the main bronchi. After deposition of the droplet, the catheter and bronchoscope were removed and the halothane anesthesia and O₂ were turned off. The endotracheal tube was removed as the dogs recovered from the anesthesia. Total anesthesia time averaged ~10 min.

Reproducibility of the methods was assessed. Repeat measurements 2 wk apart were made in each dog with the exception of the middle-aged adults. Only one set of measurements was made at this time. The animals were randomly assigned to an instillation order regardless of age group.

**Detection.** Gamma camera scintiphoto-graphs of the droplet were taken immediately upon deposition and every minute thereafter. A total observation period of 25 min was used unless material was completely cleared from the trachea in less time. Scintillation counts were recorded using a Digital Equipment Corporation PDP 11/34 computer.

**Analysis.** Droplet movement was discerned by the change in location of the droplet center as determined by computer analysis. Velocities were calculated by measuring distance moved and dividing by elapsed time.

Paired t tests were used to test for significance of the differences between measurement in individual animals. Paired t tests were also used to determine the significance of the differences between the young, middle-aged, and mature adults (dependent data within the same group of dogs). The Wilks-Shapiro test of normality, F test for variance ratio, and Student’s t test were used to test for significance of the differences between the immature and young adult dogs and mature and aged dogs (independent data among different groups of dogs).

**RESULTS**

The average clearance velocities of the radiolabeled particles for each age group are shown in Table 1. The ⁹⁹ᵐTc-macroaggregated albumin usually moved as a single discrete spot. Occasionally two spots would form, and in such cases the average velocity was taken. In all such cases the velocities of the two spots were within 20% of each other.

No significant differences were noted between first and second measurements within the same dogs. Therefore, for comparison between age groups, the first and second trials were averaged. The mean tracheal mucous velocities were 3.6 ± 0.4 (SE) mm/min for the immature dogs, 9.7 ± 0.6 mm/min for the young adults, 6.9 ± 0.5 mm/min for the middle-aged dogs, 3.5 ± 0.8 mm/min for the mature dogs, and 2.9 ± 0.5 mm/min for the aged dogs. Differences between the mean tracheal mucous velocities were significant (P < 0.05) between the immature and young adult dogs, young adult and middle-aged dogs, and the middle-aged and mature dogs. No significant differences were noted between the mature and aged dogs. In those dogs measured repeatedly over time, no correlation was found between the magnitude of tracheal mucous velocity at young adulthood vs. the rate of decline with age in individual dogs.

Figure 1 indicates that mucous velocities rose to a maximum in young adulthood and then declined with age. A function was fit to the mean velocity data. Velocity had an exponential growth with time to a maximum followed by a linear decrease. The function had the following form

$$V(t) = a(1 - e^{-bt}) - ct$$

The parameters determined from the best fit were

- $a = 11$ mm/min (maximum velocity)
- $b = 0.9/yr$ (rate of maturation, such that 95% development would be achieved in 3 yr)
- $c = 0.6$ mm/yr (rate of decline)

**DISCUSSION**

The present study demonstrated that mucous velocities in dogs increased up to young adulthood and subsequently declined with age. Goodman et al. (8), when studying the relationship of smoking history and pulmonary function, found a significantly lower tracheal mucous velocity in older (56–70 yr) nonsmokers (5.8 ± 0.38 mm/min, mean ± SE) compared with younger (19–28 yr) nonsmokers (10.1 ± 1.1 mm/min). This study demonstrated similar values in dogs. Young adult beagle dogs cleared at an average of 9.7 ± 0.6 mm/min and the mature dogs cleared at 3.5 ± 0.8 mm/min. The ages of the human subjects in the study of Goodman et al. were converted, using the method described by Mauderly and Hahn (11), to the equivalent age in dog years, and the velocity data were plotted on Fig. 1. The function for
AGE EFFECTS ON TRACHEAL MUCOCILIARY CLEARANCE IN DOGS

TABLE 1. Mucociliary clearance velocities of instilled $^{99m}$Tc macroaggregated albumin deposited in trachea of beagle dogs

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Immature (9-10 mo)</th>
<th>Young adult* (2.8-3.0 yr)</th>
<th>Middle age* (6.7-6.9 yr)</th>
<th>Mature* (9.9-9.8 yr)</th>
<th>Aged (13.7-16.2 yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
</tr>
<tr>
<td>1</td>
<td>3.0‡</td>
<td>5.4</td>
<td>13.5</td>
<td>13.3</td>
<td>7.2</td>
</tr>
<tr>
<td>2</td>
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<td>6.5</td>
<td>9.0</td>
<td>7.3</td>
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<td>3.3</td>
<td>8.8</td>
<td>8.8</td>
<td>9.1</td>
</tr>
<tr>
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<td>1.7</td>
<td>2.8</td>
<td>10.7</td>
<td>10.8</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td>6.1</td>
<td>3.4</td>
<td>6.2</td>
<td>6.8</td>
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<td>2.0</td>
<td>5.3</td>
<td>8.9</td>
<td>7.4</td>
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</tr>
<tr>
<td>7</td>
<td>2.7</td>
<td>3.6</td>
<td>10.7</td>
<td>8.6</td>
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<tr>
<td>8</td>
<td>3.5</td>
<td>1.6</td>
<td>10.2</td>
<td>14.7</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Mean ± SE
3.6 ± 0.4  9.7 ± 0.6  6.9 ± 0.5  3.5 ± 0.8  2.9 ± 0.5

* Data from same group of 8 dogs measured over time. † Only one set of measurements made at this time period. ‡ Deceased prior to measurements.

It should be noted that the specific technique used to measure mucociliary clearance might influence the observed results. The tracheal velocities measured in this paper and also by Goodman et al. (8), which both used minimally invasive techniques, were somewhat higher than those measured following inhalations by Yeates et al. (20, 21) and Foster et al. (7). Thus the possibility exists that the observations represent an age-related response to intubation or anesthetics. There are no reports of age effects on these responses. Because a consistent technique of measurement was used throughout the experiment and the measurements were made after intubation and anesthesia had ceased, it seems more likely that a true age effect on mucous velocities was responsible for the observations. This view was supported by the fact that a similar effect has been reported following inhalations. Puchelle et al. (13), when studying bronchial mucociliary clearance of inhaled $^{99m}$Tc-labeled resin particles in humans, reported a decrease of radioactivity elimination with age. Twenty five year olds had a mean mucociliary clearance (percent radioactivity eliminated in 1 h) of 34.1%, 44.6 yr olds had one of 31.3%, and 64.4 yr olds had one of 21.8%. A significant difference was noted between the 25- and 64-yr-old humans. In another study examining radioactivity clearance of chronic bronchitics compared with normal non-smokers, Puchelle et al. (14) reported that one 54-year-old person cleared 30.1% after 1 h.

The agreement between the measurements of age effects on tracheal velocities and bronchial clearance is encouraging. It is also in agreement with the correlation between tracheal and whole lung clearance observed by Yeates et al. (21) and Foster et al. (7) in humans with individuals in a normal base-line state. However, caution must also be exercised in using tracheal velocities because Foster et al. (7) also demonstrated that if a β-adrenergic stimulus was used, the response in the trachea was less than in other airways.

In our review of the effects of aging on the lungs of various species, Mauderly and Hahn (11) noted that, in animal species studied thus far, age-related changes in canine lung function are most similar to those in man. Although little change in total lung capacity occurs with age of the dog, age-related reductions are seen in such parameters as vital capacity, uniformity of gas distribution, efficiency of gas mixing, loss of elastic recoil, and alveolar-capillary gas exchange within adult animals. Mauderly (10) noted an increase to maximal values in young human adults for such measurements as vital capacity, timed forced expiratory volumes, and midexpiratory flow indexes. Thus the pattern of age-related changes in mucous velocity noted in this study is qualitatively similar to changes described for certain other pulmonary function measurements. The parallelism could reflect a maturity and integrity of the lung that is maximal in young adulthood (8). The observed changes in tracheal mucous velocities do not appear to be related to differences in tracheal dimension, as previously suggested in an interspecies comparison (5). The maximum difference in mean tracheal length was 9% and in mean tracheal surface area was 19% among the different groups. Any possible differential effects of intubation would also be minimized because tracheal lengths were
similar at all ages.

This study provided data on the same subjects studied over a period of years. There was very good interindividual reproducibility from one measurement time to another. Dogs that cleared material relatively quickly or slower at one time remained consistent over many years. Good intraindividual reproducibility has also been observed in studies of humans, though repeat studies have extended only over days or a few weeks (12, 17, 20).

Age or time is a factor in many respiratory effects of current interest. For instance, Vastag et al. (15) have presented excellent data showing a relation between mucociliary clearance impairment and pack-years of smoking. If the individual smoking histories and ages of subjects were available, a relation between bronchial clearance and age could be used to determine the effect of smoking alone. Data on age dependence could also be useful for determining the real effects of other variables such as disease processes, which also take years to develop.

Slowing of mucociliary clearance does not necessarily imply a greater susceptibility to lung disease. All the dogs in this study were clinically normal throughout the study and there was no apparent difference in health status between the slow and fast clearers. However, it seems likely that reduced velocities might indicate an increased probability of lung disease in elderly populations and might be one of the contributing factors for such observations. It has also been clearly demonstrated that mucociliary clearance is significantly slowed in chronic bronchitis (8, 18); whether this is cause or effect is unclear.

In summary, significant differences (P < 0.05) were noted in tracheal mucociliary flow in young adult and middle-aged dogs compared with immature, mature, and aged dogs. No significant difference was noted between mature and aged dogs. This pattern of change is consistent with that described for humans in studies that have been, of necessity, less well-controlled (8, 13). These changes are similar to age-related changes in some other pulmonary function parameters (10, 11).

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