In vivo evaluation of the effectiveness of bronchial thermoplasty with computed tomography

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Submitted 26 October 2004; accepted in final form 9 January 2005

In recent studies, it has been reported that the application of thermal energy delivered through a bronchoscope (bronchial thermoplasty) impairs the ability of airway smooth muscle to shorten in response to methacholine (MCh) (6, 7). The method has been termed bronchial thermoplasty. If such a technique is successful, it has the potential to serve as a therapy to attenuate airway narrowing in asthmatic subjects regardless of the initiating cause that stimulates the smooth muscle. In the present study, we have applied high-resolution computed tomography to accurately quantify the changes in airway area before and after a standard MCh aerosol challenge in airways treated with bronchial thermoplasty. We studied a total of 193 airways ranging from 2 to 15 mm in six dogs. These were divided into treated and control populations. The MCh dose-response curves in untreated airways and soon-to-be-treated airways were superimposable. In contrast, the dose-response curves in treated airways were shifted upward at all points, showing a significantly decreased sensitivity to MCh at both 2 and 4 wk posttreatment. These results thus show that treated airways have significantly increased luminal area at any dose of inhaled MCh compared with untreated airways. The work in this study thus supports the underlying concept that impairing the smooth muscle may be an effective treatment for asthma.

METHODS
This study examined the effect of treating multiple airways of six healthy mongrel dogs with bronchoscopically delivered radio frequency (RF) energy. The study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Six dogs weighing ~20 kg were anesthetized with thiopental sodium (15 mg/kg induction dose followed by 10 mg·kg⁻¹·h⁻¹ intravenous maintenance dose). After induction of anesthesia, the dogs were paralyzed with 0.5 mg/kg of succinylcholine with occasional supplemental doses as required to ensure no respiratory motion during imaging. After endotracheal intubation with an 8.0-mm-ID endotracheal tube, the dogs were placed supine and their lungs were ventilated with room air with a volume-cycled ventilator (Harvard Apparatus, Millus, MA) at a tidal volume of 15 ml/kg and a rate of 18 breaths/min. A stable depth of anesthesia was maintained by monitoring heart rate changes and eyelash reflex.

Treatment with the Alair System. Treatments were performed throughout the targeted lung region in all accessible intrapulmonary airways. The animals were treated with the Alair System (Asthmatx, Mountain View, CA) for bronchial thermoplasty, comprising a low-power RF generator and a basket catheter with four electrodes. The RF generator supplied power using temperature feedback control to maintain the target treatment setting for 10 s at each treatment site. Entire lung regions of all accessible intraparenchymal airways >2 mm in diameter and distal to the carina were treated. The left and right sides of the lungs were used to assign treatment and control regions in this study. One side of the lungs in each dog received treatments of all accessible and visible airways (quantifiable on HRCT) at a temperature setting of 75°C. The allocation of treatment and control (untreated) lung side for each dog was selected at random such that three dogs were assigned treatment to the left lung and three to the right. The untreated lung served as an internal control in each animal. Observers were blinded to treatment conditions for all HRCT measurements of airway area.

MCh challenges. On a separate day before treatment (pretreatment), 20 min after the induction of anesthesia, a dose-response curve to aerosolized MCh was initiated. The dogs received cumulative inhalation challenges of aerosol MCh (Sigma Chemical, St. Louis, MO), in concentrations of 0.3, 1, 3, 10, and 30 mg/ml. Five breaths at each dose were administered to a peak airway pressure of 15 cmH2O, held for 1 s, and then released to atmospheric pressure. Aerosol challenges were administered by a Hudson 3000 nebulizer (Hudson, Temecula, CA), driven by compressed oxygen at 10 l/min. Approximately 1 ml of solution was administered at each dose, with doses administered 5 min apart. Under test parameter conditions with an operating pressure of 50 psi and an atomization flow rate of 10 l/min, the nebulizer produces particles of mass median diameter of 3.1 μm with a geometric standard deviation of 3.2. Given such a relatively disperse particle size distribution, good central and peripheral distribution should have been achieved (9).

Recent studies have reported that the application of thermal energy delivered through a bronchoscope impairs the ability of airway smooth muscle to shorten in response to methacholine chloride (MCh) (6, 7). The method has been termed bronchial thermoplasty. If such a technique is successful, it has the potential to serve as a therapy to attenuate airway narrowing in asthmatic subjects regardless of the initiating cause that stimulates the smooth muscle. In the present study, we have applied high-resolution computed tomography to more accurately quantify the changes in airway area before and after a bronchoscope (bronchial thermoplasty) impairs the ability of airway smooth muscle to shorten in response to methacholine (MCh) (6, 7). The method has been termed bronchial thermoplasty. If such a technique is successful, it has the potential to serve as a therapy to attenuate airway narrowing in asthmatic subjects regardless of the initiating cause that stimulates the smooth muscle. In the initial evaluation of this potential therapy in a canine model, Danek et al. (7) described preliminary histological changes and responsiveness to local MCh challenge by visually estimating airway diameters. In the present study, we have applied high-resolution computed tomography (HRCT) to more accurately quantify the changes in airway area before and after a standard MCh aerosol challenge in airways treated with bronchial thermoplasty. Our results show that treated airways have significantly increased luminal area at any dose of inhaled MCh compared with untreated airways.

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after the 30 mg/ml MCh dose, the dogs received 0.2 mg/kg atropine, a dose previously shown to completely block vagal tone in the dog (4), and the HRCT scans were repeated 10 min after atropine administration.

Imaging and analysis of airways. HRCT scans were obtained with a Somatom Volume Zoom scanner (Siemens, Iselin, NJ) using a spiral mode to acquire 60 computed tomography (CT) images during an 8-s breath hold (apnea) at 137 kVp and 165 mA. The images were reconstructed as 1 mm slice thickness and a 512×512 matrix using a 125-mm field of view and a high-spatial-frequency (resolution) algorithm that enhanced edge detection, at a window level of −450 Hounsfield units and a window width of 1,350 Hounsfield units. These settings have been shown to provide accurate measurement of luminal size as small as 0.5 mm in diameter (8, 11). For repeated airway measurements in a given dog within each experimental protocol, adjacent anatomic landmarks, such as airway or vascular branching points, were defined and used to measure the airway size at the same anatomic cross sections. Using these landmarks, it was possible to correlate the location of each airway in the stacked series of CT images with that obtained from the bronchoscopic treatment location. The bronchoscopic location of each treated airway was determined by mapping the airway location with the procedure previously described in detail by Danek et al. (7).

The HRCT images were analyzed using the airway analysis module of the volumetric image and display analysis image analysis software package (Div. of Physiologic Imaging, Univ. of Iowa, Dept. of Radiology, Iowa City, IA) as previously described and validated (1, 4). The HRCT images were transferred to a UNIX-based Sun workstation. All airways with a long axis-to-short axis ratio of 1.5:1 were measured. An initial isocenter was drawn within each airway lumen, and the software program then automatically located the perimeter of the airway lumen by sending out rays in a spoke-wheel fashion to a predesignated pixel intensity level that defines the luminal edge of the airway wall. Intra- and interobserver accuracy and variability of the software program using this HRCT technique in phantoms, consisting of rigid tubes to measure known areas, has been previously shown by our laboratory (8) and by others (1) to be highly resistant to operator bias.

Data analysis. In each dog, 47–56 airways (range 1.1–16.8 mm in diameter after atropine) were identified and measured under all conditions. The mean airway areas after atropine for treated and untreated airways were compared by unpaired t-test. The completely relaxed airway after atropine was then defined as 100% (relaxed state), and airway luminal areas were expressed as a percentage of their atropine size. Airway responses to MCh were analyzed by generalized analysis of variance, with the airway area (as percentage of maximum) the dependent variable, and the multiple airways per dog, the MCh doses of MCh administered (Fig. 1). In addition, in these untreated airways and subsequently treated airways, there were no differences in the size of the airways at any MCh dose administered.

The dose-response curves to MCh in treated airways were shifted upward, indicating a significantly decreased sensitivity at both 2 and 4 wk posttreatment. Because there were no qualitative differences in the airway responses among dogs, in the subsequent figures we show the group means at each time point for treated and untreated airways.

Two weeks after bronchial thermoplasty, there was a significant difference between the treated and the untreated airways. Before MCh challenge, the mean size of the treated and the untreated airways was significantly different (108 ± 3 and 90 ± 2% of their maximum size respectively; P < 0.0001). Both the treated (P < 0.0001) and the untreated (P < 0.0001) airways 2 wk after the bronchial thermoplasty were larger relative to their pretreatment maximum size (i.e., before MCh challenge) (Figs. 1 and 2). In addition, compared with untreated airways, there were significant differences (P < 0.0001) in treated airway size 2 wk after treatment at the maximum MCh dose administered (54 ± 1%), and this decreased responsiveness was evident throughout the range of doses of MCh administered (Fig. 2).

Four weeks after bronchial thermoplasty, there was a significant difference between the treated and the untreated airways. Before MCh challenge, the mean size of the treated and the untreated airways was significantly different (95 ± 2 and 86 ± 3% of their maximum size, respectively; P = 0.02). The mean size of both the treated (P = 0.0016) and the untreated (P = 0.0006) airways remained significantly larger than at the pretreatment time (79 ± 3 and 75 ± 3%, of their maximum size, respectively; Figs. 1 and 3). There was a significant difference in the size of the airways at the maximum MCh dose administered (20 ± 1 and 34 ± 2% for the treated airways at baseline and 4 wk after treatment; P < 0.0001; Fig. 3). Similar to the 2-wk time point, this decreased responsiveness was evident throughout the range of doses of MCh administered (Fig. 3).

DISCUSSION

The results presented in this study show that bronchial thermoplasty can significantly attenuate the ability of airways...
to narrow in response to \( \text{MCh} \) for up to 1 mo after treatment. The airway size of treated airways was larger at baseline and at all doses of \( \text{MCh} \). Although our data show a slightly smaller effect at 4 wk than at 2 wk posttreatment, we do not believe that this reflects a progressive recovery from the treatment. Although we did not follow the animals beyond 1 mo, in the previous work by Danek et al. (7), dogs with a similar treatment were followed for up to 3 yr after treatment, and they found that the impaired responsiveness shown in this study at 1 mo was maintained out to 3 yr.

We did not assess the amount of smooth muscle damage histologically, but we assume that the effects were similar to those shown by Danek et al. (7) with their highest temperature treatment setting, which was also 75°C. The detailed thermodynamics of the heat transfer from the four electrodes that contact the epithelium to the airway smooth muscle is not well understood, because there has been no relevant modeling applicable to this problem. However, the decreased responsiveness to \( \text{MCh} \) is likely related to the amount of smooth muscle elimination as was previously found by Danek et al. The extent of smooth muscle damage is a very important issue, but it is very difficult to assess without sufficient numbers of the individually treated airways in histological sections. Because it is not easy to reliably identify where smooth muscle used to be in a specific histological section, one must then statistically compare the treated airways sections to a population of similarly sized untreated airways. Such a study was beyond the scope of the present work.

Although there is likely acute damage to other airway structures such as nerves or vessels from bronchial thermoplasty, we believe that it is most likely the direct action on airway smooth muscle that caused the attenuation of airway constriction. Epithelium, blood vessels, and nerves all have a good capacity for regeneration, a fact supported by direct observations of the airways. During the bronchoscopy, we did see some minor airway epithelial damage immediately after treatment. At the 2-wk measurement, however, the airways look normal, and treated airways cannot be identified visually from their epithelial surface (7). We did not perform CT scans on the animals immediately after treatment, but 2 wk after the treatment there were no gross changes in the appearance of airway or parenchymal structures on CT scans.

In these studies we chose \( \text{MCh} \) as the agonist. \( \text{MCh} \) is a direct airway smooth muscle constrictor agonist that bypasses neural mechanisms. Changes in a potentially impaired bronchial circulation may influence airway responses. As demonstrated by Blosser et al. (2), when the bronchial circulation is decreased, the degree of the airway constriction is increased. It was suggested that the bronchial circulation was important in removing the agonist agent. Thus, if the bronchial vessels were damaged by the treatment, we would have expected to see the opposite effect, i.e., increased reactivity. That we did not suggest that either there was no change in the bronchial circulation, or whatever acute damage there might have been to the bronchial circulation, it was fully recovered at the 2-wk measurement.

It may be important that we found the treated airways, before \( \text{MCh} \) challenge, to be slightly larger than under similar conditions pretreatment. One possibility to account for this might be structural alteration to other (nonmuscle) components in the airways such as collagen or other fibrous elements. Damage to these fixed structures in the airway wall from the bronchial thermoplasty could contribute to a slight dilation of the airways even without a reduction in baseline airway smooth muscle tone. However, such changes are not consistent with the results of Danek et al. (7) that showed no significant histological changes to the airways other than to the smooth muscle after a similar bronchial thermoplasty in dogs. A more likely cause of the slight dilation of the airways before \( \text{MCh} \) relates to the loss of baseline tone. There is normally a variable amount of baseline tone, which is dependent on variable amounts of smooth muscle activation (5). This fact may explain why there was also a slightly larger airway size at baseline in the untreated airways at the selected time points. However, if the muscle is reduced or impaired by bronchial thermoplasty, then not only would the ability to respond to \( \text{MCh} \) be impaired, but the normal baseline tone would also be expected to decrease.

The measurement of responsiveness we used differed from that employed by Danek et al. (7) in three important ways. First, Danek et al. visually estimated airway size by traversing the \( \text{MCh} \) application catheter tip of known size across the airway. Although this method can provide semiquantitative
estimates of airway size in all bronchoscopically accessible airways, there are obvious limitations to its accuracy. On the other hand, CT measurement of airway size has been shown to provide accurate estimates in airways as small as 0.5 mm (8, 11). One advantage of the method used by Danek et al., however, is that all airways that can be accessed with a bronchoscope can be measured regardless of the orientation of the airway. With CT imaging, one cannot accurately measure changes in small airways whose orientation is too oblique to the fixed position of the CT gantry (11), so the number of airways that can be quantified is fewer. In addition, the relatively high measurement uncertainty in the technique used by Danek et al. led them to choose a local MCh challenge dose that elicited a strong bronchoconstriction response in untreated airways. Although this allowed them to demonstrate a significant difference in responsiveness between treated and untreated airways, it limited their work to a single MCh dose delivered by local instillation. The accuracy of CT airway measurements allowed measurement of complete dose-response curves with a conventional aerosol MCh challenge.

Second, Danek et al. (7) did not reference the airway size to a fixed structural maximum. Our laboratory has previously noted that there is considerable variability in the baseline size of individual airways over the course of days or weeks (5). That this variability in baseline size of airways results from varying degrees of baseline smooth muscle tone was shown by the fact that airways fully relaxed with atropine show a stable airway size over time. Much of the variability that Danek et al. found in prechallenge airway diameter is consistent with the long-term variability that our laboratory previously observed (5). Such a change in baseline size affects the ability to interpret changes in responsiveness to MCh, especially when responsiveness is presented as a percentage of this varying baseline. In the present study, at 2 wk posttreatment, we found a slightly increased airway area in the treated airways at baseline and at all doses of MCh. In the dog, there is considerable resting airway tone (3), but because the treatment only decreases the amount of smooth muscle in the airway wall, but does not eliminate it, we observed a slight increase in airway area following treatment. Danek et al. were not able to observe an increased resting size, because their methods lacked the reliability and precision that are available with HRCT.

Third, Danek et al. (7) used a local MCh challenge, whereas we used a more conventional aerosol challenge. Although both methods can provide good estimates of airway responsiveness, the aerosol challenge method is more directly comparable agonist challenges in human subjects.

A final comment regarding potential differences between our approach and that of Danek et al. (7) relates to how functional assessments can be compared over long time periods. To study individual airways over time, it is essential to have some reference with which to normalize the degree of contraction. We know that baseline size is not reliable, because our laboratory has observed a variable degree of normal baseline tone (3). However, we have also shown that nearly all of this baseline tone can be eliminated by giving atropine, indicating its cholinergic origin. We have further verified that atropine causes maximal relaxation in the dog by showing that no further increases occur from increases in transpulmonary pressure above $\approx 7$ cmH$_2$O (4). Were there some residual tone remaining at 7 cmH$_2$O, then increasing the inflation pressure to 20 cmH$_2$O or higher would surely be able to cause some further distension. This was not observed. Of even more relevance, is the fact that we also observed that the maximally relaxed airway sizes of each airway were not significantly different on two occasions separated by up to 4 wk (3). Although we have not systematically measured the size of maximally relaxed individual airways for periods much longer than 1 mo, in a few animals we have found no changes over several months, so we believe that, in the dog, the atropine-relaxed size represents a stable structural reference. This conclusion allows us to normalize the agonist-induced constriction in each airway to this stable maximal size, whereas the approach used by Danek et al. was based on narrowing from a potentially varying baseline size.

In conclusion, we note that this potential approach of treating asthma by reducing the amount of functional smooth muscle highlights the issue as to whether there is any physiological function of airway smooth muscle (10). Clearly if there were some essential function of airway smooth muscle, then it might not be helpful to damage it. If not, however, then the approach of treating asthma by impairing the ability of smooth muscle to narrow airways, either with bronchial thermoplasty or with pharmacological approaches, may have considerable clinical promise.

ACKNOWLEDGMENTS

We thank Richard Rabold and Alexis Bierman for technical support.

GRANTS

This research was supported by National Heart, Lung, and Blood Institute Grant HL-66020.

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