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

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Brown, Robert H., William Wizeman, Christopher Danek, and Wayne Mitzner. In vivo evaluation of the effectiveness of bronchial thermoplasty with computed tomography. J Appl Physiol 98: 1603–1606, 2005; doi:10.1152/japplphysiol.01210.2004.—A recent study has 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) (Danek CJ, Lombard CM, Dungworth DL, Cox PG, Miller JD, Biggs MJ, Keast TM, Loomas BE, Wizeman WJ, Hogg JC, and Leff AR. J Appl Physiol 97: 1946–1953, 2004). 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.

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 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.

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, Millis, 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). At the completion of the scans

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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 <1.5:1 were measured. An initial isocontour 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 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).

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DISCUSSION

The results presented in this study show that bronchial thermoplasty can significantly attenuate the ability of airs
to narrow in response to MCh for up to 1 mo after treatment. The airway size of treated airways was larger at baseline and at all doses of 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 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 airway 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 MCh as the agonist. 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 suggests 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 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 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 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 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 rela-
tively 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 signifi-
cant difference in responsiveness between treated and un-
treated 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-re-
sponse 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 consid-
erable 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 labo-
atory 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 pres-
sure above ≈7 cmH₂O (4). Were there some residual tone
remaining at 7 cmH₂O, then increasing the inflation pressure to
20 cmH₂O 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 con-
clusion allows us to normalize the agonist-induced constriction
in each airway to this stable maximal size, whereas the ap-
proach used by Danek et al. was based on narrowing from a
potentially varying baseline size.

In conclusion, we note that this potential approach of treat-
ing asthma by reducing the amount of functional smooth
muscle highlights the issue as to whether there is any physio-
logical 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.

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