Ipratropium Decreases Airway Size in Dogs by Preferential M₂ Muscarinic Receptor Blockade In Vivo

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Background: Two major groups of drugs are available to prevent bronchoconstriction: beta-agonists and muscarinic blocking agents. Ipratropium is the most commonly used anticholinergic agent to treat chronic obstructive pulmonary disease. The authors studied anti-muscarinic agents to determine if they are as effective bronchodilators as beta-adrenergic agents and if not to identify the mechanism of their reduced effectiveness.

Methods: Six anesthetized dogs were studied using high-resolution computed tomography to measure changes in the cross-sectional area of conducting airways induced by cumulative doses of ipratropium with and without gallamine, a selective M₂ muscarinic receptor blocker, and after metaproterenol.

Results: Metaproterenol dilated the airways and ipratropium constricted the airways. Ipratropium in concentrations of 0.01 and 0.1 mg/ml constricted the airways to 22 ± 2% and 20 ± 3% of control, respectively (P < 0.01), whereas larger concentrations caused bronchodilation. After complete blockade of the M₂ receptors by pretreatment with intravenous gallamine, the bronchoconstrictor effect of ipratropium was abolished, and ipratropium dilated the airways by 16 ± 8% and 27 ± 10% of pre-gallamine baseline after doses of 0.01 and 0.1 mg/ml, respectively (P < 0.01).

Conclusion: Low-dose ipratropium can decrease airway size by the initial, preferential blockade of neuronal M₂ muscarinic receptors, whereas a larger dose of ipratropium blocks M₂ muscarinic receptors on airway smooth muscle, resulting in bronchodilation. (Key words: Pharmacology, bronchodilators: ipratropium; metaproterenol. Lung, airways: bronchodilation. Measurement technique: high resolution computed tomography.)

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Received from The Johns Hopkins Medical Institutions, Baltimore, Maryland. Submitted for publication April 21, 1996. Accepted for publication June 16, 1996. Supported by National Institutes of Health grant HL02795 and the American Lung Association of Maryland.

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Induction of anesthesia and tracheal intubation activates airway reflexes and causes airway constriction in many patients with asthma and chronic obstructive pulmonary disease. Baseline airway tone and reflex bronchoconstriction in the conducting airways are mediated by the parasympathetic nerves in the vagi. These nerves release acetylcholine onto M₂ muscarinic receptors on the airway smooth muscle, which leads to contraction of the muscle and bronchoconstriction. Pharmacologic blockade of parasympathetic pathways or surgical transection of the vagi causes bronchodilation, demonstrating that airway smooth muscle is tonically contracted by the parasympathetic nerves. In addition to the M₂ receptors on the airway smooth muscle, M₂ muscarinic receptors exist presynaptically on the parasympathetic nerves of the lung. Stimulation of these M₂ receptors inhibit the acetylcholine release by as much as 80%, whereas pharmacologic blockade potentiates release of acetylcholine fivefold.

Two major groups of drugs are available to prevent reflex-mediated bronchoconstriction. Beta-agonists stimulate beta-adrenergic receptors in the lung and cause bronchodilation, whereas muscarinic antagonists block muscarinic receptors on the airway smooth muscle and cause bronchodilation. Ipratropium is the most commonly used anticholinergic agent to treat chronic obstructive pulmonary disease, but surprisingly its effectiveness is limited. Ipratropium increases acetylcholine release in human and guinea pig tracheal tissue with electrical field stimulation, and lower concentrations of ipratropium potentiated vagal nerve stimulation induced bronchoconstriction in guinea pigs. Furthermore, paradoxical bronchoconstriction has been reported in humans immediately after inhalation of ipratropium. Thus we determined whether ipratropium is as effective as metaproterenol at dilating airways with baseline tone and if not to identify the mechanism that reduces ipratropium’s effectiveness.
Because parasympathetic nerves predominantly innervate conducting airways larger than 1 mm in diameter, high-resolution computed tomography (HRCT) was used in anesthetized dogs to measure changes from baseline in the cross-sectional area of these innervated airways induced by cumulative doses of ipratropium, in the presence and absence of gallamine, a selective M2 receptor antagonist. Metaproterenol, a beta-adrenergic agonist agent, was administered for comparison.

Materials and Methods

This study was approved by the Johns Hopkins Animal Care and Use Committee. Six mongrel dogs were anesthetized with thiopental (15 mg/kg induction dose followed by 10 mg⋅kg⁻¹⋅h⁻¹ intravenous maintenance dose). After paralysis induced by succinylcholine (0.5 mg/kg), the trachea was intubated with an 8.5-mm (inner diameter) endotracheal tube and the lungs were ventilated with a volume-cycled ventilator (Harvard Apparatus, Millis, MA) with 100% oxygen at a tidal volume of 15 ml/kg and a rate of 18 breaths/min.

Visualizing the Airway Area

High-resolution computed tomography scans were obtained using a Somatom Plus scanner (Siemens, Iselin, NJ) using a 1-s scan time, 137 kVp, and 220 mA. Fifty to 55 contiguous scans were obtained, starting approximately 5 mm above the origin of the right upper lobe bronchus from the trachea and proceeding caudally using 1-mm table feed and 2-mm slice thickness. The dogs were aneic at function residual capacity during the scans (approximately 2 min). Images were reconstructed using a high-spatial frequency (resolution) algorithm that enhances edge detection, and at a window level of -450 Hounsfield units (HU) and window width of 1,550 HU. These window settings previously were shown to allow optimal lung resolution. All airways visualized in approximately cross-section from the scan plane (long-to-short-axis ratio less than 1.5:1) were measured under all experimental conditions. For repeated image analysis within each experiment and across experiments on different days, proximal anatomic landmarks, such as airway or vascular branching points, were defined on the control state HRCT image. The same airways in a given animal were then analyzed on images matched by these anatomic landmarks.

Evaluation of Airways

The HRCT images were transferred as 16-bit data images to a UNIX-based work station and reduced to eight-bit images, which were then analyzed using the airway analysis module of the Volumetric Image and Display Analysis software package (Department of Radiology, University of Iowa, Iowa City, IA). To measure airway areas, the operator drew an isocontour within the lumen of the airway. The software then automatically located an isocontour perimeter of the airway lumen by sending out rays like the spokes of a wheel to a predesignated pixel intensity level that defined the lumenal edge of the airway wall. The length of the rays was set at 6 pixels. The software program used an algorithm for edge detection based on the “full-width-half-maximum” principle. The edge of the wall was defined by the program by the points along the rays where the pixel intensity changed to one half its maximum through the wall. All full and partial pixels (full pixel size was 0.23 mm² with our settings) within the adjusted isocontour were counted and represented the airway area. Intra- and interobserver accuracy and variability of the software program using this HRCT technique in phantoms consisting of rigid tubes to measure known areas is highly resistant to operator bias.

Protocol

Each dog served as its own control. On separate days in random order, at least 1 week apart, the dogs received increasing doses (2, 4, 8, and 16 puffs) of either ipratropium (18 μg/puff; Boehringer Ingelheim, Ridgefield, CT; n = 6) or metaproterenol (650 μg/puff; Sandoz Pharmaceutical, East Hanover, NJ; n = 3) by metered-dose inhaler. The metered-dose inhalers were administered by activating the canister “puffs” through a y-connector attached to the endotracheal tube and activated at the beginning of inspiration.

Because results from the first series suggested that ipratropium constricted the conducting airways, we repeated the ipratropium challenges with freshly prepared solutions for aerosol administration of ipratropium bromide (Sigma Chemical Co., St. Louis, MO) in saline rather than from the commercial metered-dose inhaler canisters. On separate days in random order, at least 1 week apart, the dogs received ipratropium aerosols in increasing concentrations (0.01, 0.1, 1.0, and 10 mg/ml) with (n = 2) and without (n = 6) 10 mg/kg intravenous gallamine pretreatment (Sigma Chemical Co.). Ipratropium was diluted in 0.9% saline.
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(the pH of the lowest concentration of ipratropium, 0.01 mg/ml, was 6.79) and administered as an aerosol using a Hudson nebulizer (model 3000; Temecula, CA) connected to the endotracheal tube for five breaths. Each breath was standardized to a peak pressure of 15 cm H₂O and maintained for 2 s. One milliliter of each concentration of solution was nebulized using compressed oxygen at a flow rate of 5 l/min. The mass median diameter of the particle was 4.2 μm, with a geometric standard deviation of 3.5. High-resolution computed tomography scans were obtained before drugs were administered (control) and repeated after each inhalation. The dogs were apneic for approximately 2 min while scans were acquired, and then normal ventilation was resumed. Scan acquisitions occurred at approximately 10-min intervals.

Analysis

Fifteen airways could be visualized in cross section from the scan plane (long-to-short-axis ratio less than 1.5:1) under all experimental conditions and were matched and measured in each of the dogs. The airway diameters ranged from 2.9 mm to 21.2 mm, with 79% of the airways within the range of 3 to 8 mm. The airway areas as a percentage change from control were analyzed by two-way analysis of variance controlling for the individual dogs (between-dog variability) and for the multiple airway measurements per dog (within-dog variability), and with Bonferroni correction for multiple pairwise comparisons of means. Differences were considered significant at P < 0.05.

Results

Data represent mean ± SEM percentage change in airway area from control for all measured airways. Metaprotenerol was more effective than ipratropium at smaller doses, whereas the two drugs were equally effective at larger doses. Two puffs of metaprotenerol did not significantly change airway area, whereas 4, 8, and 16 puffs increased airway area by 17 ± 7%, 25 ± 8%, and 42 ± 9%, respectively (P < 0.01). In contrast, ipratropium administered by metered-dose inhaler produced airway constriction at the smaller doses. Two and four puffs of ipratropium significantly decreased the airway area by 25 ± 2% and 25 ± 3% of baseline, respectively (P < 0.01). Eight puffs of ipratropium decreased airway area by 9 ± 6%. Bronchodilation was only apparent after 16 puffs of ipratropium (30 ± 12% increase from control, P < 0.01).

Similarly, ipratropium aerosol produced a significant bronchoconstriction at the two lower doses and bronchodilation at the highest dose. Ipratropium aerosol at concentrations of 0.01 and 0.1 mg/ml decreased airway area by 22 ± 2% and 20 ± 3%, respectively (P < 0.01). At a concentration of 1 mg/ml, ipratropium caused no significant change in airways caliber (1 ± 4% decrease from control), whereas at the highest concentration (10 mg/ml) ipratropium caused a significant bronchodilation and increased airway area by 51 ± 9% (P < 0.01; figs. 1 and 2).

After complete blockade of the M₁ muscarinic receptors by pretreatment of intravenous gallamine, airway size decreased significantly by 13 ± 2% of control (P < 0.01; fig. 2) and abolished the bronchoconstrictor response to ipratropium. In the presence of gallamine, even the lowest concentration of ipratropium aerosol (0.01 mg/ml) dilated the airways by 16 ± 8% of control airway area before gallamine pretreatment (P < 0.01; fig. 2). Concentration of 0.1 mg/ml ipratropium further diluted the airways by 27 ± 10% of control (P < 0.01; fig. 2).

Discussion

This study shows that metaprotenerol was more effective than ipratropium at smaller doses and equally
effective at larger doses at dilating canine airways. Furthermore, small doses of ipratropium, either by metered-dose inhaler or aerosol, constricted the conducting airways in vitro. However, after blockade of the M₂ receptors by intravenous gallamine, ipratropium aerosol dilates the airways.

In the absence of gallamine, ipratropium probably blocked both the M₂ and M₃ muscarinic receptors. Although ipratropium is considered to be a nonselective muscarinic antagonist, and thus should block M₂ and M₃ receptors equally, it has been shown to have a slightly greater affinity for the neuronal M₂ receptors than the M₃ receptors on the airway smooth muscle. At smaller doses, ipratropium increased baseline airway tone by the initial, preferential blockade of neuronal M₂ muscarinic receptors, resulting in increased release of acetylcholine. Larger doses of ipratropium blocked M₃ muscarinic receptors on airway smooth muscle, overcoming this increased acetylcholine release, and resulting in bronchodilation in the dogs. The largest doses used caused bronchodilation comparable to the maximal dilation seen with complete vagal blockade after intravenous atropine and inhalation anesthesia.

The ipratropium-induced airway constriction was abolished by blocking the neuronal M₂ receptors with intravenous gallamine, a selective M₂ receptor antagonist. We selected a dose of gallamine that would completely block the M₂ muscarinic receptors. In the presence of gallamine, all doses of ipratropium aerosol, even the smallest concentration, dilated the airways to more than control size. Therefore, in gallamine-pre-treated dogs, ipratropium dilated the airways by directly blocking the M₁ receptor.

If inhaled ipratropium and intravenous gallamine both caused constriction of the airways by blocking neuronal M₂ receptors, then both agents might have been expected to produce similar degrees of airway constriction. However, gallamine has a significantly greater affinity for M₂ than the M₃ receptors, whereas ipratropium has only a slightly greater affinity for the M₂ than the M₃ receptors. Furthermore, it is unlikely that the effective doses on the M₂ receptors were comparable. The drugs were given by different routes and thus it is not reasonable to compare the magnitude of the effects. More importantly, they both caused qualitatively similar airway constriction.

Although we used succinylcholine to paralyze the dogs, it is unlikely that the succinylcholine potentiated the cholinergic responses we observed. Unlike gala-

Fig. 2. Airways response to ipratropium aerosol with and without 10 mg/kg intravenous gallamine pretreatment as the percentage of control. The ipratropium aerosol was administered in doses of 0.01, 0.1, 1, and 10 mg/ml. The open squares represent the percentage change in airway lumenal area (mean ± SEM) from control after each dose of ipratropium aerosol alone. The closed circles represent the percentage change in airway lumenal area from control (before gallamine administration) in the presence of intravenous gallamine pretreatment after ipratropium aerosol administration. Ipratropium aerosol alone significantly decreased airway lumenal area after the 0.01- and 0.1-mg/ml concentrations (P < 0.01) and significantly increased lumenal area only after the 10-mg/ml concentration. Pretreatment with intravenous gallamine significantly constricted the airways (P < 0.01). However, intravenous gallamine prevented the ipratropium-induced initial airway constriction. After intravenous gallamine pretreatment, ipratropium aerosol significantly dilated the airway lumenal area, even at the lowest dose compared with control (P < 0.01).
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mine, succinylcholine has not been shown to potentiate vagally induced bronchoconstriction.9

Vagal innervation and muscarinic receptors are located predominantly in conducting airways 3 to 8 mm in diameter.16-23 Tracheal intubation stimulates these conducting airways.1,2,29 In this study, 79% of the airways measured were 3 to 8 mm in diameter, which are in the middle of the range of airways that can be measured by HRCT. For airways in this size range, HRCT can detect changes in conducting airways that cannot be measured by global lung function assessment.3,16,25 The decreased ability of global measurements to detect changes in conducting airways could account for the lack of observed airway constriction after ipratropium administration in previous studies.30-32

Although lung volume was not measured in this study, changes in lung volume at FRC during 2 min of apnea would be small. Using similar methods, we determined that calculated changes in lung volume after histamine induced airway constriction were small.23 Although the total time to acquire scans was 2 min, some airways were scanned at the beginning and others at the end of the 2-min period. Because the direction of scanning was always the same (cranial to caudal direction), each measured airway was scanned at the same time during the apneic period. In addition, the period of apnea was the same for the dogs given low-dose ipratropium with and without gallamine, and thus even if small lung volume changes occurred they could not account for the differences seen in the airway responses.

The time for ipratropium bronchodilating activity can be long. Maximal bronchodilation after ipratropium administration can take as long as 90 min.33 However, we were interested in the immediate response of the airways to ipratropium because preoperative administration of a bronchodilator commonly occurs immediately before induction of anesthesia and tracheal intubation. Furthermore, the reports of paradoxical bronchoconstriction in humans occurred immediately after inhalation of the ipratropium.15-17 Therefore we measured airway caliber within 10 min after drug administration, which was the earliest we could complete the challenges and acquire the HRCT scans. Although the maximum effect of ipratropium on airways can take as long as 90 min, 50% of the maximal response occurs within 3 min in humans.23 Although we observed airway constriction within 10 min of the administration of ipratropium, whether the airways subsequently dilate over time requires further study.

Controversy exists about the amount and variability of drug delivered to the airways through a metered-dose inhaler, and is thought to be as little as 6% of the published dose per activation.35,36 More recently, various spacer devices have been used to increase the efficiency of drug delivery.35 We did not use a spacer in the present study. Although the exact amount of drug delivered to the dogs’ airways was not measured in this study, the airway responses to the increasing doses of ipratropium were similar whether the drug was administered by metered-dose inhaler or aerosol. In addition, the airways dilated in a dose-response manner in response to the metaproterenol administered as an MDI, which is consistent with increased delivery of the drug with increased numbers of activations of the metered-dose inhaler.

Our findings of ipratropium-induced airway narrowing in dogs are consistent with many reports of airway narrowing in humans. However, our results do not support the mechanisms previously proposed as the cause of this ipratropium-induced airway constriction in humans, which include nonspecific irritation of the airways,16 preservatives in the inhaler,14 toxicity of the solution,15-17 acidity of the solution,17 or increased mucus viscosity.37 Because gallamine does not block the afferent limb of the vagal reflex arc, abolition of the ipratropium-induced constrictor effect in the presence of intravenously administered gallamine makes nonspecific irritation an unlikely explanation of our results. Although the preservative ethylenediamine tetracetic acid is no longer used, chlorofluorocarbons are used as propellants for metered-dose inhalers. However, we also observed airway constriction when ipratropium was administered in an isotonic saline solution and no bronchoconstriction after metaproterenol was administered using a metered-dose inhaler. High acidity of the ipratropium solution would not account for the bronchoconstriction, because the lowest pH of the ipratropium aerosol solution was 6.79, and a recent study evaluating the effect of a preservative-free and pH-adjusted solution in persons with asthma found no differences in the responses of patients with stable or acute asthma using ipratropium with or without pH adjustment of the solutions.38 Finally, other investigators found no increased mucus viscosity after ipratropium administration.39 In addition, we found no evi-
dence in the present study of mucus plugging on the HRCT scans in the airways we visualized.

Kil and associates also had limited effectiveness with ipratropium at preventing the increase in total lung resistance after intubation in patients with chronic obstructive pulmonary disease. This may be due to the dose administered. A larger dose may have been more effective at blocking the reflex-induced increase in lung resistance.

Ipratropium increases acetylcholine release in human and guinea pig tracheal tissue with electrical field stimulation, and lower concentrations of ipratropium-potentiated vagal nerve stimulation induced bronchoconstriction in guinea pigs. Ipratropium can increase baseline airway tone by the initial, preferential blockade of neuronal M1 muscarinic receptors, resulting in increased release of acetylcholine and airway narrowing. However, larger doses of ipratropium block M1 muscarinic receptors on airway smooth muscle, thus overcoming this increased acetylcholine release, and result in bronchodilation in the dogs. Although the airway innervation of dogs and humans are not identical, both species have substantial parasympathetic innervation in the airways. Therefore these data suggest that a larger dose than that currently recommended should be studied in patients with obstructive lung disease to prevent this initial increased airway tone and promote the bronchodilating effects of ipratropium.

The authors thank Dr. Allison Fryer for suggestions and assistance, Drs. Carol Hirshman, Wayne Mitzner, and Elias Zerhouni for ideas and support, Beatrice Mudge for assistance in the radiology laboratory, Richard Rabold for technical assistance, and Dr. Eric Hoffman for the use of the Volumetric Image and Display Analyses software program.

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