Nebulized Lidocaine Prevents Airway Inflammation, Peribronchial Fibrosis, and Mucus Production in a Murine Model of Asthma


ABSTRACT

Background: Evidence suggests that nebulized lidocaine is beneficial in asthma therapy, but to what extent and the mechanisms underlying this effect remain poorly understood. The aim of this study was to assess the impact of lidocaine treatment using a murine model of allergic asthma characterized by expression of pivotal features of the disease: inflammation, mucus production, and lung remodeling.

Methods: A/J mice sensitized with ovalbumin were treated with inhaled lidocaine or vehicle immediately after ovalbumin intranasal challenges. Lung function, total and differential leukocytes in bronchoalveolar lavage fluid, peribronchial eosinophil density, interleukin (IL)-4, IL-5 and eotaxin-1 levels, epithelial mucus, collagen, extracellular-matrix deposition, matrix metalloproteinase-9 activity, and GATA-3 expression were evaluated. Between five and eight animals per group were used.

Results: Inhaled lidocaine inhibited ovalbumin-induced airway hyperreactivity to methacholine, and accumulation of lymphocytes, neutrophils, and eosinophils in bronchoalveolar lavage fluid 24 h after the last allergen provocation. Lidocaine administration also prevented other pathophysiological changes triggered by ovalbumin in lung tissue, including peribronchial eosinophil and neutrophil infiltration, subepithelial fibrosis, increased content of collagen and mucus, matrix metalloproteinase-9 activity, and increased levels of IL-4, IL-5, IL-13, and eotaxin-1. Furthermore, inhaled lidocaine inhibited lung tissue GATA-3 expression in ovalbumin-challenged mice. We also demonstrated that lidocaine inhibited the expression of GATA-3 in ovalbumin-stimulated T cells in vitro.

Conclusions: Inhaled lidocaine prevents eosinophilic inflammation, overproduction of mucus, and peribronchial fibrosis in a murine model of asthma, and impaired airway hyperreactivity, possibly by inhibiting allergen-evoked GATA-3 expression and the subsequent up-regulation of proinflammatory cytokines.

What We Already Know about This Topic

- Nebulized lidocaine is beneficial in asthma therapy, but to what extent and the mechanisms involved remain poorly understood.

What This Article Tells Us That Is New

- In a murine model, nebulized lidocaine prevented eosinophilic inflammation, overproduction of mucus, and peribronchial fibrosis, and decreased airway hyperreactivity possibly by inhibiting allergen-evoked GATA-3 expression and the subsequent up-regulation of proinflammatory cytokines.

Allergic asthma is a chronic inflammatory disorder of the airways driven by T cells and Th2 cytokines in which lung-infiltrating eosinophils are suggested to play a pivotal part. Recurrent and persistent asthma leads to airway remodeling, which is characterized by subepithelial fibrosis and marked increase in the levels of collagen and other extracellular matrix proteins. In addition, excessive secretion of mucus and hyperplasia/hyper trophy of smooth muscle strongly contribute to thickening of airway walls, hyperreactivity.
ity, and airflow limitation, which require appropriate therapeutic intervention and can be fatal.7,8

Bronchospasm (the clinical expression of severe airway hyperreactivity) is also a frequent life-threatening perioperative event, which can be triggered by pharmacologic and/or mechanical factors.7 Remarkably, uncontrolled asthma is one of the most common pathologic features underlying perioperative bronchospasm, occurring in up to 9% of asthmatic patients during general anesthesia.9 Inhaled lidocaine has been shown to attenuate bronchospasm in response to a variety of stimuli while evoking airway anesthesia, but interestingly, topical airway anesthesia and blockade of bronchial hyperreactivity are two independent effects, as previously demonstrated.10 Although the benefit is not immediate, glucocorticoids have also been used as an alternative to prevent perioperative bronchospasm because they decrease airway inflammation.9

Asthmatics usually effectively prevent their symptoms with inhaled steroid antiinflammatory drugs. However, some patients require treatment with high doses of oral glucocorticoids, which leads to adverse effects, and 1 or 2% of asthma patients are entirely corticosteroid-insensitive, pointing to the need for alternative antiinflammatory therapy.11-13 Serendipitously, while measuring eosinophil-active cytokines in bronchoalveolar lavage (BAL) effluent from asthmatic patients, Ohnishi et al.14 identified lidocaine (used for topical anesthesia during the BAL procedure) as a potent inhibitor of cytokine-evoked survival and activation of eosinophils. The observation that lidocaine, in addition to preventing bronchospasm, could mimic the antieosinophil properties of glucocorticoids strongly stimulated investigations on the putative application of lidocaine in asthma therapy.14,15

Several approaches have shown the efficacy of nebulized lidocaine in reducing the use of oral glucocorticoids in patients with moderate and severe corticosteroid-dependent asthma.15-18 Notably, lidocaine effectively treated a pregnant patient whose symptoms of severe asthma could not be controlled by conventional medications, including glucocorticoids.19 These findings suggest that nebulized lidocaine may be a useful therapeutic alternative for severe and corticosteroid-resistant asthma. However, this assumption is controversial because lidocaine failed to improve pulmonary function in recent studies involving patients with mild or moderate asthma and patients with more severe asthma.20

Models of lung allergic inflammation based on rats21 and guinea pigs22 have been used effectively to confirm the capacity of nebulized lidocaine to prevent allergen-induced inflammatory infiltrate and airway hyperreactivity. Nevertheless, the effect of this treatment on tissue remodeling and hypersecretion of mucus has not been explored. Consequently, the goal of the current study was to investigate the effect of lidocaine on ovalbumin-sensitized A/J mice, an asthma-prone strain.23 We have also addressed the effects of lidocaine on allergen-induced expression of the transcription factor GATA-3.

Materials and Methods

All protocols and experimental procedures involving animals were approved by the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil; license number, CEUA L-034/09).

Immunization, Antigen Challenge, and Treatment Protocols

Male A/J mice, obtained from the Oswaldo Cruz Foundation Breeding Center, were immunized subcutaneously on day 0 and boosted by intraperitoneal injection 14 days later with 50 μg ovalbumin (grade V; Sigma-Aldrich, St. Louis, MO) adsorbed to 5 mg of Al(OH)3 in 200 μL of 0.9% NaCl (sterile saline). Intranasal ovalbumin challenges (25 μg/25 μL) were administered on days 19 and 20 under halothane volatile anesthesia (Cristália, São Paulo, Brazil). Sensitized control mice were challenged only with vehicle. Just after allergen provocation, mice were placed in a plexiglas chamber (45 × 28 × 29 cm) and treated by exposure to an aerosol of lidocaine (0.25, 0.5, and 1%) or an equal volume of sterile saline for 30 min using an air-driven nebulizer (model NS I-210/12; Indústria de Aparelhos Biomédicos, São Paulo, Brazil) at a flow rate of 10 L/min.

Airway Responsiveness to Methacholine

Airway hyperreactivity (AHR) was determined as changes of airway function after aerosolized methacholine 24 h after the last ovalbumin challenge. Transpulmonary resistance and compliance were assessed in anesthetized (nembutal 60 mg/kg, intraperitoneal), tracheostomized, and mechanically ventilated mice. Mice were monitored in a whole-body plethysmograph with a pneumotachograph connected to a transducer. Air flow and transpulmonary pressure were recorded with a Buxco Pulmonary Mechanics Computer (Buxco Electronics, Sharon, CT). Changes in resistance and compliance were taken at baseline, after aerosolized phosphate-buffered saline (PBS), and each increasing concentration of methacholine provocation (3, 9, and 27 mg/ml) as previously reported.24

Measurement of Pulmonary Inflammation

One hour after the last methacholine bronchoprovocation, mice were killed by anesthetic overdose (sodium pentobarbital, 500 mg/kg, intraperitoneal). Cells were recovered from the airway lumen through BAL. Airways were lavaged twice with 0.75 ml PBS containing 10 mM EDTA via a tracheal cannula. BAL fluid was centrifuged (400 × g, 10 min, 4°C). Cell pellets were resuspended in 250 μL PBS for further enumeration of leukocytes in a Neubauer chamber by means of a light microscope (BX50; Olympus, Center Valley, PA) after dilution with Türk solution (2% acetic acid). Differential cell counts were carried out on May–Grunwald–Giemsa-stained cytospin preparations under oil immersion objective to determine the percentage of macrophages, lymphocytes, neutrophils, and eosinophils.
Measurement of Peribronchial Fibrosis and Mucus Production

The left lung was fixed in Milloning buffer solution (pH 7.4) with 4% paraformaldehyde to preserve the pulmonary architecture. Samples were embedded in paraplast media (Sigma-Aldrich) and 4 μm-thick sections were stained with hematoxylin and eosin stain and a combination of Gömöri trichrome stain (Trichrome Stain LG Solution; Sigma-Aldrich). The area of peribronchial trichrome staining (i.e., between the alveolar septum and airway epithelium of 10 distal airways by lung section) was outlined and quantified for the total deposition of the extracellular matrix (including collagen fibers). The evaluation was made in an image analyzer system (Image-Pro® Plus, 4.1; Media Cybernetics, Houston, TX) using digitalized images obtained from a light microscope at a magnification of ×400. Results were expressed as extracellular matrix deposition area (μm²).

Histologic sections were stained for eosinophils and neutrophils with Llewellyn’s Sirus Red (Direct Red 80, CI 35780; Aldrich, Milwaukee, WI). Eosinophil and neutrophil infiltrates were evaluated around the airway as well as between the bronchial epithelium and adventitia through an integrating eyepiece (10⁴ per μm² of total area). Determinations were made in six randomly selected fields at a magnification of ×1,000 and expressed as eosinophils and neutrophils/unit area (μm²).

The amount of lung collagen was measured in frozen lungs homogenized in Tris-HCl 0.05 M, 1 M NaCl containing protease inhibitor (Hoffmann-La Roche, Basel, Switzerland) at a pH of 7.4. Total soluble collagen were extracted overnight at room temperature and measured by the Sircol (Worthington, Lakewood, NJ) at a wavelength of 535 nm. Data were expressed as collagen (μg) per mg of tissue.

Detection of Matrix Metalloproteinases

Sodium dodecyl sulfate-substrate zymography electrophoresis was undertaken using a previously described method with modifications. Briefly, supernatant aliquots from lung samples (20 μg) were loaded onto a 10% polyacrylamide gel containing 0.1% gelatin under nonreducing conditions. After electrophoresis, gels were washed in renaturation buffer 2.5% Triton X-100 (in 0.05 M Tris-HCl), for 1 h to remove the sodium dodecyl sulfate. Substrate digestion was undertaken by incubating the gel in 0.05 M Tris-HCl, pH 7.6, containing 0.005 M CaCl₂ and 0.15 M NaCl at 37°C for 20 h. Gels were stained with 0.5% Comassie Brilliant Blue R250 (Bio-Rad, Hercules, CA). Gelatinolytic activity was visualized as clear bands against a dark background. The molecular weight of the gelatinolytic band was estimated by comparison with prestained molecular-weight markers.

Histologic sections (4 μm) were stained with hematoxylin and eosin stain and periodic acid-Schiff stain (Periodic Acid–Schiff Stained System, Sigma-Aldrich) for measuring mucus production. Total neutral mucus substance area was evaluated in the respiratory epithelium of 10 distal airways by lung section using digitalized images at a magnification of ×400 and an image analyzer system (Image-Pro® Plus Media Cybernetics, Bethesda, MD). Results were expressed as total mucus substance area (μm²).

Quantification of Cytokines and Eotaxin

After recovery of BAL fluid from the airway lumen, 24 h after the last ovalbumin challenge, the thorax was opened and a cannula inserted through the right ventricle to allow the pulmonary vasculature to be flushed at low pressure with saline to remove the blood pool of cells. The left lung lobes were removed, immediately frozen in liquid nitrogen, and stored at −80°C. Commercial enzyme immunoassay kit (ELISA) kits were used for the measurement of cytokine and chemokine proteins in whole-lung homogenates and cell-free supernatants. Briefly, lung tissue was homogenized on ice using a Tissue Tearor (μH Homogenizer; Omni International, Kennesaw, GA) for 30 s in 1 ml PBS containing 0.05% Triton X-100 and a protease inhibitor cocktail (Hoffmann-La Roche, Basel, Switzerland). The resulting supernatants were isolated after centrifugation (10,000 × g, 15 min, 4°C). Samples were quantified using commercially available kits (interleukin [IL]-4, IL-5, IL-13; DuoSet; R&D Systems, Minneapolis, MN) and eotaxin-1 (R&D Systems), according to manufacturer instructions.

Western Blotting Analyses

Pooled cervical, axial, and inguinal lymph node cells (10⁶/well) from naïve DO11.10 T cell receptor transgenic mice were treated with ovalbumin (0.5 mg/ml) and exposed to lidocaine (300 μM). Treatments lasted for 24 h at 37°C in an atmosphere of 5% CO₂. After this incubation, cells were lysed with 100 μl of a solution containing Triton X-100 (0.1%) and Complete Protease Inhibitor Cocktail 1X (Roche Diagnostics, Indianapolis, IN). Cell suspensions were sonicated for 10 min and centrifuged at 3,000 × g for 10 min at 4°C. Supernatants were retrieved and stored at −20°C until use. Lung tissue was homogenized as described above and stored at −20°C until evaluation.

For western blotting analyses, samples with equal protein concentration (50 μg) were separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose membranes. Nonspecific binding was blocked overnight with 5% nonfat dry milk in Tris-buffered solution containing 0.1% Tween-20. Membranes were incubated with the primary antibody (anti-human GATA-3; Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h. After washing with Tris-buffered solution, membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (at 1:3,000; Calbiochem, San Diego, CA) for 1 h at room temperature. After three washes of membranes with Tris-buffered solution (10 min each), protein bands were detected using an electrochemiluminescence kpl plus western blotting detection system with subsequent exposure to X-ray film (Kodak, Rochester, NY). Western blotting images were analyzed by densitometry using Gelplot analysis macros in Scion Image Software, version 4.03 (Scion Corporation, Frederick, MD).
Analyses of Apoptosis and T-cell Cycles by Flow Cytometry

Naive cells obtained from the lymph nodes of DO11.10 T cell receptor transgenic male mice (10^6 cells/well) were stimulated with ovalbumin (0.5 mg/ml). Cells were treated with lidocaine (100, 300, and 600 µM) for 72 h at 37°C in an atmosphere of 5% CO2. DNA content and fragmentation of retrieved cells was analyzed by permeabilizing and staining cells with propidium iodide (PI) as described.24 Briefly, cells were stained with 75 mM PI in the presence of the nonionic detergent NP-40 (Calbiochem) for 15 min. Analyses of DNA content and calculation of the percentage of hypodiploid cells were done by collecting 10,000 or 20,000 events for proliferation and apoptosis analyses, respectively, in a FACSCalibur flow cytometer for analyses using CellQuest software (BD Biosciences PharMingen, San Diego, CA).

Statistical Analysis

Data analyses were performed with a statistical software package (Prism version 5.0, Graph-Pad Software, San Diego, CA). Data were expressed as mean ± SD or median and interquartile range. All tests were carried out using one-way ANOVA, except those concerning the airway hyperreactivity data, which were analyzed using a two-way ANOVA with post hoc Bonferroni correction. Concerning all the other normally distributed data, analyses were done with ANOVA followed by the Student-Newman-Keuls test. When variables were nonnormally distributed, data were analyzed using a nonparametric Kruskal–Wallis test, with a post hoc Dunn test. Statistical differences were considered significant if P < 0.05 (two-tailed tests).

Results

Effect of Nebulized Lidocaine on Allergen-induced Leukocyte Recruitment into the Lung

As compared with control mice challenged with saline, ovalbumin provocation of sensitized A/J mice caused a significant increase in total leukocyte numbers detected in BAL fluid (fig. 1A) 24 h after the last provocation. Such an increase in cell counts was accounted for elevation in the numbers of macrophages (fig. 1B), lymphocytes (fig. 1C), neutrophils (fig. 1D), and eosinophils (fig. 1E). Nebulization with lidocaine (0.5–1%) for 30 min immediately after ovalbumin provocations inhibited all of these changes, except the change concerning increased macrophage numbers (figs. 1A–E). Histologic evaluation of the lungs of saline-challenged mice (fig. 2A) and ovalbumin-challenged mice (fig. 2B) revealed strong peribronchial eosinophilic inflammatory infiltration after allergen provocation that was clearly sensitive to lidocaine (fig. 2C). Quantitative morphometric analyses of lung sections showed that nebulized lidocaine markedly inhibited the infiltration of eosinophils (fig. 2D) and neutrophils (fig. 2E).
Effect of Nebulized Lidocaine on Allergen-induced AHR

To access the effect of lidocaine on alterations in lung function, we undertook invasive measurements of lung resistance and dynamic compliance responses to inhaled methacholine (3–27 mg/ml) 24 h after the last allergen provocation. Anti-gen challenge with ovalbumin led to AHR as determined by increased lung resistance and reduced lung dynamic compliance responses after methacholine (3–27 mg/ml) challenge as compared with control mice challenged with saline (figs. 3A and B, respectively). Nebulized lidocaine (0.5% and 1%) significantly attenuated AHR in ovalbumin-challenge A/J mice, whereas 0.25% lidocaine did not.

Effect of Nebulized Lidocaine on Allergen-induced Mucus Production

To determine if lidocaine could exert protective effects on allergen-induced mucus exacerbation, lung histologic sections were harvested 24 h after the last provocation and stained with periodic acid-Schiff. The representative photomicrograph of saline (fig. 4A) shows no mucus production, whereas ovalbumin-challenged A/J mice (fig. 4B) exhibited goblet-cell hyperplasia and mucus hypersecretion (arrowhead) within the airway epithelia. One-percent lidocaine significantly inhibited ovalbumin-induced mucus secretion (fig. 4C). Quantitative morphometric analyses showed that treatment with lidocaine (0.25 to 1%) concentration-dependently inhibited mucus exacerbation, reaching a blockade value of 75% at the highest dose (fig. 4D).

Effect of Lidocaine on Allergen-induced Subepithelial Fibrosis and Up-regulation of Collagen Levels and Matrix Metalloproteinase-9 Activity in Lung Tissue

Airway remodeling is a major cause of airflow obstruction in asthma. To assess if nebulized lidocaine could affect allergen-evoked lung remodeling, lung histologic sections were harvested 24 h after the last allergen provocation and stained with Gömöri trichrome, a classic staining for extracellular matrix components. Biochemical quantification of collagen and matrix metalloproteinase 9 activity in lung-tissue samples were also assessed. Figure 5 shows a representative lung photomicrograph from mice challenged with saline, displaying a predictable normal extracellular matrix profile. In contrast, ovalbumin-challenged mice had increased extracellular matrix deposition in peribronchiolar areas (fig. 5B) that were abrogated by 1% lidocaine (fig. 5C). Quantitative morphometric analyses of these sections revealed that lidocaine (0.25 to 1%) concentration-dependently prevented peribronchial fibrosis (fig. 5D). These findings were confirmed by collagen quantification in lung tissue samples using the Sircol technique. Ovalbumin-challenged mice presented a 2-fold increase in the amount of lung collagen as compared with that of control mice.
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Fig. 3. Effect of nebulized lidocaine on allergen-induced changes in transpulmonary resistance and dynamic compliance. Airway responsiveness was measured by changes in airway resistance (A) and dynamic compliance (B) induced by increasing concentrations of methacholine 24 h after the last ovalbumin or saline challenge. Ovalbumin-sensitized mice were divided into five groups: saline-challenged (n = 10), ovalbumin-challenged (n = 10), ovalbumin-challenged treated with 0.25% lidocaine (n = 7), ovalbumin-challenged treated with 0.5% lidocaine (n = 7), and ovalbumin-challenged treated with 1% lidocaine (n = 9). Data are expressed as mean ± SD. *P < 0.05 as compared with the saline group; †P < 0.05 as compared with the ovalbumin-challenged group; &P < 0.05 as compared with the ovalbumin-challenged treated with 0.5% lidocaine group. OVA = ovalbumin.

in saline-challenged mice (fig. 5E), and lidocaine (0.25 to 1%) affected this response. This finding lent support to the results obtained with Gömöri trichrome-stained tissues. Furthermore, using gelatin zymography, we found that nebulized lidocaine also inhibited the increased enzymatic activity of matrix metalloproteinase-9 detected in the lung tissues of ovalbumin-challenged mice (figs. 5F and G).

Effect of Nebulized Lidocaine on Allergen-evoked Up-regulation of Cytokines and Chemokine Levels in Lung Tissue

Levels of the Th2 cytokines IL-4, IL-5, and IL-13, as well as of the chemokine eotaxin-1, were increased in the lung tissue of ovalbumin-challenged mice as compared with those of the negative control group (fig. 6). Administration of lidocaine abrogated the increased concentrations of cytokines and chemokine assessed (figs. 6A–D).

Effect of Nebulized Lidocaine on Allergen-evoked GATA-3 Expression in Lung Tissue and Lymph Node T Cells

GATA-3 is essential for the induction of Th2 differentiation and initial expression of cytokines. To investigate the capacity of lidocaine to inhibit GATA-3 expression, the levels of this transcription factor were monitored in treated and untreated mice by western blotting analyses of lung tissue. Expression of GATA-3 was significantly increased in whole-lung extracts from ovalbumin-challenge mice as compared with that of control mice challenged with saline, and administration of lidocaine concentration-dependently reduced the increased expression of GATA-3 (fig. 7A). Lymph node T cells obtained from DO11.10 T cell receptor transgenic mice had increased GATA-3 expression after exposure to ovalbumin in vitro, and were also sensitive to 300 μM lidocaine pretreatment in vitro (fig. 7B).

Effect of In Vitro Lidocaine Treatment on T Cells

Lymph node cells exposed to allergen provocation in the presence or absence of lidocaine were permeabilized, stained with PI, and analyzed by flow cytometry. There was an increase in the percentage of proliferation and a decrease in the percentage of apoptotic cells when they were exposed to ovalbumin for 72 h (fig. 8). Treatment of ovalbumin-activated cells with lidocaine (300–600 μM) significantly decreased the percentage of proliferating T cells (fig. 8A) and increased the percentage of apoptosis (fig. 8B).

Discussion

The local anesthetic lidocaine has shown beneficial effects in asthma therapy, but its mode of action and extent of effects remain unclear.18,19 In the present study, we investigated the effects of lidocaine on pivotal pathologic changes triggered by allergen challenge in a murine model of asthma. We demonstrated that nebulized lidocaine prevents AHR, eosinophilic inflammatory infiltrates, mucus secretion, subepithelial fibrosis, collagen deposition in lungs, and matrix metalloproteinase 9 activity triggered by repeated ovalbumin intranasal challenges in ovalbumin-sensitized mice. This effect is associated with decreased levels of proinflammatory Th2 cytokines and eotaxin-1 in parallel with abrogation of GATA-3 expression. Our findings support the hypothesis that inhaled lidocaine can be efficacious for the treatment of asthma, particularly for the improvement of lung function, tissue remodeling, and mucus hypersecretion.

Goblet-cell hyperplasia and peribronchial fibrosis are the main components of airway remodeling, and contribute to the development of AHR and the increase in severity and mortality in bronchial asthma.5,6 Several studies in patients15–19 and animal models21,22 have shown that nebulized lidocaine has beneficial effects on the decline in lung function and bronchial inflammation in asthmatic conditions. However, the effect of this treatment on tissue remodeling and mucus hypersecretion has not been explored. In the current study, we addressed this matter using a murine model of allergic inflammation based on strain A/J mice, which possess a genetic asthma-prone background and show unique sensitivity to changes in lung remodeling after repeated intranasal allergen provocations.23

We noted that two daily consecutive ovalbumin intranasal provocations in ovalbumin/alum-immunized mice was suffi-
cient to induce changes in lung resistance and elastance, eosinophilic inflammatory infiltration, airway remodeling, and mucus secretion 24 h postchallenge. These findings suggest that, in spite of being a short-term model of asthma, it can be useful for replicating the major features of the disease in humans (including those of the chronic allergic reaction). Under these conditions, the inflammatory response was marked by a bronchoalveolar accumulation of leukocytes, which was accounted for by increased levels of polymorphonuclear eosinophils (451-fold) and neutrophils (46-fold), as well as lymphocytes (20-fold) and macrophages (2-fold). Histologic assessment of inflammatory infiltrates indicated increased numbers of eosinophils (36-fold) and neutrophils (3-fold) in the peribronchial area, emphasizing the eosinophilic profile of this response.

Because of the prevalence and suggested importance of eosinophils in asthma conditions, new antiasthma therapies based on targeting eosinophils and their products are in development. One of the major reasons for lidocaine to be considered is because it down-regulates Th2 cytokine-induced activation and survival of eosinophils by causing apoptosis of these cells in a mechanism unrelated to cytotoxicity or blockade of sodium channels. Remarkably, in human eosinophils, this effect can be obtained at concentrations ranging from 10 to 1,000 μM, easily achieved in the airways with conventional nebulizers. In our model, as administered after allergen provocation, at days 19 and 20 postsensitization, nebulized lidocaine (1%) inhibited ovalbumin-evoked accumulation of eosinophils, neutrophils, and lymphocytes in the airway lumen, and also significantly abrogated subepithelial infiltration of eosinophils and neutrophils. More importantly, and unique to the present study, lidocaine prevented ovalbumin-induced peribronchial fibrosis and mucus hypersecretion as attested by histologic quantitative evaluations. Furthermore, a marked increase in lung collagen production and matrix metalloproteinase-9 activity occurred in line with airway thickening, which were accordingly abrogated by lidocaine treatment. Metalloproteinases are crucial for extracellular matrix degradation, and the lidocaine effect upon matrix metalloproteinase-9 is particularly relevant because this enzyme is up-regulated in the sputum and BAL fluid of asthmatics and participates in various aspects of asthma pathogenesis, including accumulation of inflammatory cells, collagen synthesis, and airway remodeling.

**Fig. 4.** Effect of nebulized lidocaine on mucus production. Photomicrographs of periodic acid-Schiff stain of lung tissue from saline control (A), ovalbumin-challenged (B), and ovalbumin-challenged treated with 1% lidocaine mice (C). Histologic examinations of lung tissue were carried out 24 h after the last challenge. Photomicrographs were taken of representative airways showing goblet-cell hyperplasia and mucus production (purple color, arrowheads) with an original magnification of ×400. Quantitative assessment of mucus production (D) was carried out in lung sections by morphometric analyses as described in Materials and Methods. Ovalbumin-sensitized mice were divided into five groups: saline-challenged (n = 8), ovalbumin-challenged (n = 8), ovalbumin-challenged treated with 0.25% lidocaine (n = 7), ovalbumin-challenged treated with 0.5% lidocaine (n = 7), and ovalbumin-challenged treated with 1% lidocaine (n = 8). Data (periodic acid-Schiff-positive area, pixel/μm²) are expressed as the mean ± SD. +P < 0.05 as compared with the saline group; *P < 0.05 as compared with the ovalbumin-challenged group; &P < 0.05 as compared with the ovalbumin-challenged treated with 0.5% lidocaine group. BR = bronchioles; OVA = ovalbumin; PAS = periodic acid-Schiff.
Studies using genetically modified mice specifically lacking eosinophils have been instrumental in demonstrating a causative relationship between these cells and airway remodeling and mucus secretion in models of chronic allergic inflammation. Eosinophils are recruited into the lungs and activated under the influence of chemokines such as eotaxin-1 and -2 produced by airway epithelial cells, as well as Th2-type cytokines such as IL-5. Although the effector role of eosinophils remains debatable, mounting evidence suggests that these cells are implicated in airway remodeling because they release fibrogenic factors such as IL-13, transforming growth factor β, vascular endothelial growth factor, and matrix metalloproteinase-9. The absence of eosinophils leads to a reduction in T lymphocyte numbers and Th2 responses in models of acute and chronic allergenic inflammation. More recent studies revealed that pulmonary eosinophils are linked to the recruitment of Th2-cytokine-secreting T cells via generation of CCL17 and CCL22. Thus, it is conceivable that lidocaine might mediate its inhibitory effect on airway remodeling and mucus secretion in part by inhibiting the survival and activation of eosinophils.

In asthmatics, airway inflammation is associated with cellular and structural changes that result in thickening of the airway wall, airflow limitation, and AHR. Our data revealed a close correlation on the protective effect elicited by lidocaine upon inflammation and remodeling changes, as well as with the blockade of the accompanying AHR, which is in line with the assumption that inflammation and airway remodeling contribute to AHR. Interestingly, at the lowest dosage (0.25%), lidocaine failed to influence allergen-induced AHR, in spite of significantly reducing changes in airway thickening and mucus secretion, under conditions in which the increased levels of neutrophils were left unchanged. These results might indicate a role for neutrophils.

Fig. 5. Effect of nebulized lidocaine on lung subepithelial fibrosis, total collagen, and matrix metalloproteinase-9 activity after ovalbumin provocation in mice. Photomicrographs are representative of lung histologic sections (stained with Gomori trichrome stain) obtained from saline-challenged (A), ovalbumin-challenged (B), and ovalbumin-challenged mice treated with 1% lidocaine (C). Quantitative assessment of fibrotic changes (D) was carried out in lung sections. Ovalbumin-sensitized mice were divided into five groups: saline-challenged (n = 8), ovalbumin-challenged (n = 8), ovalbumin-challenged treated with 0.25% lidocaine (n = 7), ovalbumin-challenged treated with 0.5% lidocaine (n = 7), and ovalbumin-challenged treated with 1% lidocaine (n = 8). Quantitative assessment of total collagen (E) was carried out in lung samples by Sircol collagen assay. Ovalbumin-sensitized mice were divided into five groups: saline-challenged (n = 7), ovalbumin-challenged (n = 7), ovalbumin-challenged treated with 0.25% lidocaine (n = 5), ovalbumin-challenged treated with 0.5% lidocaine (n = 5), and ovalbumin-challenged treated with 1% lidocaine (n = 6). Quantitative assessment of matrix metalloproteinase-9 activity (F) was carried out in lung samples by gelatin zymography. Ovalbumin-sensitized mice were divided into five groups: saline-challenged (n = 5), ovalbumin-challenged (n = 6), ovalbumin-challenged treated with 0.25% lidocaine (n = 5), ovalbumin-challenged treated with 0.5% lidocaine (n = 5), and ovalbumin-challenged treated with 1% lidocaine (n = 5). The insert (G) shows a representative gelatin zymogram of matrix metalloproteinase-9 lung activity obtained from the respective groups. Data from fibrosis changes and total collagen are expressed as the mean ± SD, and data from matrix metalloproteinase-9 are expressed as median and interquartile range. *P < 0.05 as compared with the saline group; **P < 0.05 as compared with ovalbumin-challenged mice; ***P < 0.05 as compared with ovalbumin-challenged mice treated with 0.25% lidocaine; &&P < 0.05 as compared with ovalbumin-challenged mice treated with 0.5% lidocaine. BR = bronchioles; MMP-9 = matrix metalloproteinase-9; OVA = ovalbumin.
and their products in this lung-airway dysfunction. In fact, severe asthma and corticosteroid-resistant asthma feature a neutrophil-dependent component. Based on these findings, it is tempting to speculate that a dual effect of lidocaine on eosinophils and neutrophils may favor the efficacy of this treatment in controlling asthma.

Inflammatory cells propagate inflammation and remodeling changes in asthma via secretion of Th2 cytokines and chemokines. Our findings revealed that lidocaine nebulization markedly inhibited the ovalbumin-induced increased levels of IL-4, IL-5, IL-13, and eotaxin-1 in lung tissue. This observation suggested that the beneficial effects of lidocaine treatment may derive from its ability to simultaneously impair the generation of these key proinflammatory mediators.

The transcription factor GATA-3 is expressed in T cells, eosinophils, mast cells, and basophils. It has been found to be of particular importance in the differentiation of naïve Th cells toward the Th2 phenotype, and to play a pivotal part in cytokine gene expression in T cells. Furthermore, its expression is increased in the BAL cells of asthmatics after allergen provocation. In our model, we observed a strong increase in pulmonary GATA-3 expression after allergen provocation, which led us to assess the effect of lidocaine in this phenomenon. We demonstrated that nebulized lidocaine concentration-dependently suppressed pulmonary GATA-3 expression compared with ovalbumin-challenged mice without treatment. Furthermore, in in vitro settings, we found that 300 μM lidocaine suppressed ovalbumin-induced GATA-3 expression in lymph node T cells recovered from DO11.10 T cell receptor transgenic mice under conditions in which the survival and proliferative response of these cells were only slightly reduced. Taken together, these findings suggest that down-regulation of GATA-3 in inflammatory cells such as T cells may contribute to the antiasthma properties of lidocaine.

Recent studies have emphasized the therapeutic potential of a new series of lidocaine analogues that were synthetized and screened for reduced local anesthetic activity. These investigations strongly suggest that the use of nonanesthetic lidocaine analogues, such as JMF2-1, might be a means of achieving the antispasmodic and antiinflammatory effects of lidocaine without the anesthetic effect. This is an issue of considerable importance because one of the drawbacks of using lidocaine in asthma is the recognized airway irritation activity, which correlates in a causative manner with the local anesthetic potency of these compounds.

Finally, we recognize some important limitations of this study. First, significant bronchial pathophysiologica
do exist between mouse and human airways. Therefore, it would be relevant to confirm our findings in asthmatics, bearing in mind that nebulized lidocaine has already been proved to be an effective therapy in several patients with moderate and severe asthma.15–19,44 A second point concerns the fact that we have used a short- instead of a long-term murine model of asthma, which may better reproduce the features of chronic asthma. Lastly, a third limitation concerns the narrow range of lidocaine concentrations employed (0.25 to 1%), because it would be of interest to clarify whether higher concentrations might provide additional efficacy. Nevertheless, the study’s prevailing rationale concerning this matter was the avoidance of toxic plasma concentrations of lidocaine.

In conclusion, these findings reveal that nebulized lidocaine prevents airway eosinophilic inflammation, bronchial hyperreactivity, and pulmonary remodeling by simultaneous depletion of Th2 cytokines such as IL-4, IL-5, and IL-13, as well as eotaxin-1, in a mechanism closely related to the down-regulation of GATA-3 expression. These results reinforce the prospect of further exploiting lidocaine and/or lidocaine analogues for asthma therapy and drug development.
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References

2. Shalaby KH, Martin JG: Overview of asthma; the place of the T cell. Curr Opin Pharmacol 2010; 10:218–25

Anesthesiology 2012; 117:580–91

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Landry’s Huang-Di et l’Acupuncture

The Club Français de la Médaille released this 68-mm-diameter medal of silvered copper alloy in 1972. As designed by Annette Landry (born 1907), the obverse features a bust of China’s Huang Di (left), who lived, at least in myth, from 2698 –2598 BCE. When this legendary “Yellow Emperor” was not inventing the first compass or the first calendar, he was establishing the principles of Chinese medicine. One particular Chinese medical practice, acupuncture, is illustrated by the five thin needles protruding from the patient’s face on the reverse aspect of the medal (right). The medal pictured is number 55 of only 100 minted.

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