Effect of Inhaled Heparin on Methacholine-Induced Bronchial Hyperreactivity*

Berrin Ceyhan, MD; and Turgay Celikel, MD, FCCP

Although heparin is used as an anticoagulant, its biologic function has remained unclear since the 1920s. Glycosaminoglycan heparin possesses multiple noncoagulant properties, including anti-inflammatory actions, and it is possible that heparin may inhibit airway hyperreactivity. Thus, the purpose of the present investigation was to study the effect of inhaled heparin on methacholine-induced bronchoconstriction. Thirteen subjects (7 women, 6 men) with mild asthma were included in the study. Bronchial provocation tests were performed in a single-blind, crossover, randomized order and repeated 45 min after placebo or aerosolized heparin inhalation (1,000 U/kg). The heparin inhibited bronchoconstriction induced by methacholine. In the methacholine challenge test, heparin treatment resulted in an increase in the mean PD20 over placebo: 5.26 ± 4.80 mg/mL vs 10.57 ± 5.72 mg/mL (p<0.0002). These data suggest that inhaled heparin may have an inhibitory role on methacholine bronchial challenge, possibly via a direct effect on smooth muscle.

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IP₃ = inositol 1,4,5-triphosphate

Key words: bronchial hyperreactivity; inhaled heparin; methacholine

Our understanding of the mechanisms contributing to the pathogenesis of bronchial asthma has increased substantially over the past decade, but mast cell products, heparin and related proteoglycans, have not received much attention. Studies dating back to the 1920s have reported that heparin can inhibit the features of anaphylaxis. Clinical reports have shown that heparin can modulate allergic responses in man, both in skin and in the respiratory system.1 It has been suggested previously that aerosolized heparin may have beneficial effects in alleviating asthma symptoms, although no definite bronchodilating activity has been observed.2 Others have found the short-term administration of heparin to patients with asthmatic attacks is of no benefit.3 However, it now seems that heparin and related proteoglycans have a far greater role in the pathogenesis of asthma than previously thought. Recently it has been observed that heparin acts as a specific blocker of inositol 1,4,5-triphosphate (IP₃) receptors and inhibits IP₃-mediated calcium release in various cell types, including vascular smooth muscle, liver cells, cerebellum, and airway smooth muscle.4-8 Heparin has also been shown to possess anti-inflammatory and anticomplement activity, as well as to regulate the activity of mast cell tryptase.9-12

It is therapeutically attractive to attempt to modulate the pathophysiologic mechanisms of asthma with exogenous heparin. It has been shown that heparin attenuated the bronchoconstrictor response and immediate cutaneous reaction to antigen in allergic sheep, but failed to block the bronchoconstrictor effects of histamine and carbachol.13,14 Inhalation of 20,000 U of heparin did not provide significant protection against antigen-induced bronchoconstriction in human subjects.15 The same dose of inhaled heparin did inhibit the acute cutaneous reaction and bronchoconstriction due to allergens in allergic subjects.16 Moreover, inhaled heparin prevented exercise-induced asthma.17 However, whether heparin modulates bronchial hyperreactivity induced by methacholine is not known.

The purpose of this investigation was to study the effect of inhaled heparin on bronchial hyperreactivity using the methacholine provocation test.

METHODS

The investigation was designed to study the effect of heparin on bronchial hyperreactivity. A total of 13 nonsmoker subjects (7 women, 6 men) with a history of mild asthma and a documented bronchoconstrictor response to methacholine were included in the study.

Before entry, subjects were screened with histories, physical examinations, pulmonary function tests, including prebronchodilator and postbronchodilator spirometries, complete blood cell counts, serum IgE levels, skin prick tests, and chest radiographs.

None of the subjects had any history of respiratory tract infection or acute attack within 3 months before entering the study. Subjects were not excluded if they were receiving antiasthmatic drugs. However, they were required to have been receiving a stable dosage for 3 months and to continue the same dosage of all medications over the course of the study. Treatment with β₂-ag-
onist inhalers was withheld for at least 24 h before each study day. Written informed consent was obtained from each subject before the study. The criteria to define the mild asthma were as follows: low frequency of symptoms (no more often than one to two times a week); peak expiratory flow rate >80% predicted with <20% variability; minimal or no evidence of airway obstruction on spirometry; and not requiring regular therapy except for short periods of time.

Pulmonary function was assessed with a spirometer (Sensormedics, S3513, Calif). Each data point was the average of at least three reproducible measurements (variability <5%). Their baseline forced expiratory volume in 1 s (FEV₁) was >70% of predicted values and no patient was receiving corticosteroids or theophylline. One patient had been receiving ketotifen therapy for 3 months and another patient had been receiving ketotifen and terfenadine treatment for 1 month. All visits to the laboratory were carried out at the same time of day. To induce bronchial provocation, the nebulizer was attached to a dosimeter (Mediprom FDC 58, France) that consisted of a breath-activated solenoid valve and a source of compressed air (pressure, 20 psi). When triggered by the subject’s inspiratory effort, the solenoid valve was set to remain open for 0.4 s. After the baseline FEV₁ was measured, the subjects inhaled five breaths of saline solution diluent, and the measurements were repeated after a 2-min interval. Subjects inhaled the aerosolized solutions in five breaths from end-tidal volume to full inspiratory capacity via a mouthpiece. Starting from 0.125 mg/mL concentration, the subsequent methacholine dilutions were increased in a doubling manner, the test being stopped when the FEV₁ fell by at least 20% from the value recorded after normal saline solution. At the end of each experiment, the subjects took two inhalations of albuterol to reverse any residual bronchoconstriction.

Commercial heparin sodium (injection USP, in bacteriostatic injection water, Roche) was used undiluted as a aerosol with a concentration of 5,000 USP U/mL. The heparin was administered at a dose of 1,000 U/kg of body weight (maximal total dose, 60,000 U). The nebulized volume of the solution was between 8 and 12 mL and was given during a 20 to 30-min period. The solution of heparin was administered as a constant-flow aerosol during tidal breathing. The nebulizer provided an aerosol with a mass median aerodynamic diameter of 1 to 4 μm (Voyage nebulizer, Mefar, Italy). A placebo solution or heparin in a single-blind, crossover, randomized design was given to patients. Each subject underwent a standardized methacholine challenge test 45 min after placebo or heparin solution inhalation. The visits were separated by at least 7 days.

Data are expressed as mean±SD. A paired t test was used to compare the effect of heparin on methacholine PD20 values with that of placebo. A p value of less than 0.05 was considered to indicate statistical significance.

### Table 1—Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>13</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr (range)</td>
<td>30±11 (17-56)</td>
</tr>
<tr>
<td>Duration of asthma, yr (range)</td>
<td>7±7 (6 mo-23 yr)</td>
</tr>
<tr>
<td>Baseline spirometry</td>
<td></td>
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<tr>
<td>FEV₁, L (pred %)</td>
<td>3.44±1.24 (96.7±17.1)</td>
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<tr>
<td>FVC, L</td>
<td>4.12±1.21</td>
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<td>FEV₁/FVC, %</td>
<td>80.7±10.6</td>
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<tr>
<td>FEF25-75, L/s</td>
<td>3.39±1.67</td>
</tr>
<tr>
<td>Bronchodilator response, %</td>
<td>18.3±3.45</td>
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</tbody>
</table>

**FIGURE 1.** Changes in methacholine PD20 values after pretreatment with aerosolized heparin as compared with placebo.

### Results

The clinical characteristics of the patients are summarized in Table 1. Two of the 13 patients were taking ketotifen, one of them terfenadine, and the others albuterol metered-dose inhalation as circumstances required. Nine of them had positive skin prick tests. In all, 14 subjects were enrolled in the study, but one subject was withdrawn from the study following an episode of acute bronchospasm after inhalation of 10,000 U of heparin. In this patient, the test was stopped and the FEV₁ returned to baseline level following administration of 200 μg of inhaled albuterol.

There was no significant difference in baseline values of FEV₁ between study days. Inhaled heparin resulted in an increase of the methacholine PD20 value from 5.26±4.80 mg/mL to 10.57±5.72 mg/mL (p<0.0002). All subjects but one showed decreased airway hyperreactivity after heparin inhalation (Fig 1).

Eight of the 13 patients had mild headaches during heparin inhalation, but this resolved 1 to 2 h later.

### Discussion

The results of the present investigation show that inhaled heparin inhibits methacholine-induced bronchoconstriction. This suggests that heparin has a direct effect on airway smooth muscle.

The mechanism by which heparin attenuates asthma is not clear. The heparin molecule possesses multiple noncoagulant properties such as anti-inflammatory and antimplement activity, modulation of various proteases, and regulation of mast cell tryptase. Heparin can inhibit lymphocyte activation, trafficking, and delayed hypersensitivity responses that are lymphocyte driven. Heparin also has an effect on both eosinophils and neutrophils.
Furthermore, heparin inhibits the increased vascular permeability induced by a wide range of agonists acting via specific receptors located on the vascular endothelial cells.22 The cationic peroxidases, such as major basic protein and eosinophil peroxidase, are neutralized by the highly anionic heparin; thus, heparin inhibits the epithelial damage induced by some of these cationic proteins.23,24 It has been suggested that endogenous heparin may be released to limit the extent of eosinophil recruitment into sites of allergic inflammation and to limit the extent of tissue damage induced by cationic proteins.25 It is therefore plausible that endogenous heparin may be a natural "anti-asthmatic" molecule. An increase in levels of endogenous "heparin-like material" has also been found in the plasma of allergic patients. Furthermore, adults with asthma seem to have less calcification of major arteries than age- and sex-matched controls, and this difference has been attributed to higher levels of circulating heparin-like material.26

Commercial heparin may have multiple sites of action of asthma. The commercial heparin used in this study contained benzyl alcohol as a preservative. This preservative has been reported to cause relaxation of acetylcholine-induced contraction of canine airway smooth muscle under a dose-dependent fashion in vitro, whereas purified heparin had no relaxant effect.27 However, it has been demonstrated that the inhibitory effect of commercial heparin against antigen-induced bronchoconstriction was not related to the benzyl alcohol preservative in the sheep.13 It has also been shown that the inhibitory effect of commercial heparin was not related to its mucopolysaccharide structure or anionic charge by the failure of dextran sulfate and de-N-sulfated heparin to modify the antigen-induced bronchoconstriction.13 The anti-inflammatory activity of heparin has not been related to its anticoagulant properties. It has been shown that plasma partial thromboplastin time was not prolonged by inhaled heparin,13,17 and heparin that depleted anticoagulant activity inhibited delayed hypersensitivity.19 These observations suggest that the inhibitory effect of commercial heparin on methacholine-induced bronchoconstriction was not due to inhibition of coagulation factors and benzyl alcohol preservative. In our study using commercial heparin, the dose and lag time between heparin and challenge test were similar to those in previous studies on human subjects.17 For practical reasons, we were unable to use benzyl alcohol in the placebo preparation.

There are several reports about the effect of inhaled heparin on various bronchial challenge tests. Heparin attenuated antigen-induced bronchoconstriction in a dose-dependent fashion: 100, 300, and 1,000 U/kg of heparin causing 8, 55, and 91% inhibition of specific lung resistance in sensitized sheep, respectively.13 In the same study, authors reported the inhibitory effect of heparin on bronchoconstrictor responses induced by compound 48/80, which is a polyamine and causes nonimmunologic, and non-cytolytic mast cell degranulation and the failure of heparin on histamine- and carbachol-induced bronchoconstriction.13 However, the effect of inhaled heparin on antigen challenge in human subjects remains controversial. O'Donnell et al.15 in a study employing 20,000 U of inhaled heparin, were unable to demonstrate any reduction in the bronchoconstriction induced by a single-dose antigen challenge in human subjects with asthma. Neither the immediate nor the late response was attenuated.15 Others have demonstrated that the same dose of inhaled heparin inhibited bronchoconstriction caused by inhaled dust mite extract in allergic subjects with asthma.16 Ahmed et al reported that inhaled heparin (1,000 U/kg) prevented exercise-induced asthma without influencing histamine-induced bronchoconstriction, and attenuated postexercise decrease of the specific airway resistance more significantly than placebo and cromolyn.17 These authors suggested that the effect of heparin was related to a modulation of mediator release rather than to a direct effect on smooth muscle. We were unable to find any published report about the effect of inhaled heparin on methacholine-induced bronchoconstriction in human subjects. We believe this is the first report of heparin inhibiting nonspecific bronchial hyperreactivity. The mechanism by which heparin attenuates methacholine-induced bronchoconstriction is not clear. This could be due to a direct effect of heparin on smooth muscle, as it has been shown previously that the bronchoconstriction induced by methacholine results from direct stimulation of the smooth muscle.28,29 In contrast to our observations, Ahmed et al.30 reported that heparin (1,000 ng/mL) failed to inhibit acetylcholine-induced tracheal smooth muscle contraction in tissue culture. The discrepancy between these findings and ours may be due to the difference between human studies and in vitro studies.

In our study, inhaled heparin attenuated methacholine-induced bronchial hyperreactivity. The mechanism involved in the control of bronchial hyperreactivity by heparin has been studied little and is poorly understood. Heparin deserves further investigations in large number of subjects to provide further insight into the pathophysiologic state of asthma. Heparin may also be of clinical importance and may form the basis of novel therapeutic approaches.

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