Montelukast protects against bradykinin-induced bronchospasm

To the Editor:

Bradykinin is a potent bioactive peptide synthesized *de novo* during inflammatory processes and is considered to play an important role in bronchial asthma. In fact, bradykinin has a potent bronchoconstrictor effect in asthmatic subjects, produces several features of asthma when administered to animals, and has been implicated in allergen-induced bronchoconstriction.[1] In spite of its well-documented role in bronchial asthma, the exact way in which bradykinin elicits its activity is still debated. Some evidence suggests that bradykinin exerts its effects by inducing the release of tachykinins[2] that, in turn, induce bronchoconstriction through the production of cysteinyl leukotrienes (cys-LTs), although it is not clear yet what cellular sources and through which mechanism tachykinins cause the release of leukotrienes.[3] Interestingly, it has also been demonstrated that leukotrienes, on their own, induce the release of tachykinins, establishing a vicious circle that might mediate several features of asthma.[4]

Furthermore, *in vitro* studies have also shown that bradykinin induces the release of lipoxygenase metabolites from human lung fibroblasts and from rat lung tissue.[5,6] More recently, Shin et al[7] have shown that bradykinin, inducing the production of 12-lipoxygenase metabolites, might activate the capsaicin-sensitive vanilloid receptor, exciting sensory nerve terminals.

These findings suggest that bradykinin might exert its activities in airways by promoting, either directly or indirectly, the release of leukotrienes, contributing to the development and maintenance of bronchial inflammation and airway hyperresponsiveness. According to these observations, we hypothesized that bradykinin-induced bronchoconstriction in asthmatic patients is indirectly caused by the release of leukotrienes, and this mechanism could explain some of the antiasthmatic and anti-inflammatory effects of leukotriene antagonists.

The study consisted of 2 distinct phases and was carried out in 18 asthmatic patients, with disease severity ranging from mild to moderate classified on the basis of the criteria of the National Heart, Lung, and Blood Institute/World Health Organization Workshop on the Global Strategy for Asthma. Patients were nonsmokers, and all were atopic as defined by positive skin prick test responses to one or more of 6 common aeroallergens. At the beginning of the study, all subjects were asymptomatic, with a FEV_1 greater than 70% of predicted value. None had received oral corticosteroids, theophylline, antihistamines, or sodium cromoglycate within the preceding 4 weeks. Inhaled bronchodilatators and steroids were discontinued for at least 12 hours and 4 weeks, respectively, before each visit to the laboratory. The study was approved by the local ethic's committee, and all subjects provided written informed consent.

During the first phase of the study, subjects undertook concentration-response studies with inhaled methacholine, followed after 3 hours by a bradykinin challenge in the absence of any drug treatment to determine baseline PC_{20} values. On this occasion, as well as on the other study days, the 3-hour interval between the 2 challenges warranted a complete recovery of FEV_1 to baseline value after methacholine challenge. During the second phase, patients attended the laboratory on 2 separate occasions at least 4 days apart to undertake concentration-response studies with methacholine and bradykinin after receiving either oral montelukast or matched placebo in a double-blind, placebo-controlled, crossover design. To ensure an adequate drug level and achieve a better saturation of cys-LT1 receptors, we studied the effect of the drug, administering 2 tablets of 10 mg of montelukast 3 hours before starting the challenges. Urine for analysis of leukotriene E_4 (LTE_4) levels was collected from 16 of the 18 enrolled patients before and 2 hours after each challenge on each study day. After collection, 20-mL aliquots of urine were frozen at −80°C until the assay was performed. Urinary LTE_4 levels were measured with a commercially available enzyme immunoassay (Cayman Chemical Company, Ann Arbor, Mich) and expressed as picograms of immunoreactive LTE_4 per millimoles of creatinine in each urine sample.

Pretreatment and posttreatment baseline values of FEV_1 before bronchial challenge were compared among and within study days by means of 2-way ANOVA and, where appropriate, were followed by a Neumann-Keuls test for specific means comparisons. PC_{20} methacholine and bradykinin values after treatment with placebo and montelukast were logarithmically transformed to normalize their distribution and then compared by means of a Student *t* test for paired data. The PC_{20} values, after a logarithmic conversion, were expressed as geometric means ± SE.

There was no significant difference in mean ± SE baseline values of FEV_1 among any of the study days. Neither was there any difference between baseline FEV_1 values before methacholine and bradykinin challenges on each study day. Administration of montelukast did not cause any significant change in FEV_1 from baseline. Inhaled methacholine and bradykinin in the absence of any drug treatment produced a concentration-related bronchospasm with geometric mean PC_{20} values of 0.62 mg/mL (geometric mean + SE = 0.78; geometric mean − SE = 0.50) and 0.17 μg/mL (geometric mean + SE = 0.23; geometric mean − SE = 0.13), respectively. Placebo administration did not produce any significant change in methacholine and bradykinin responsiveness in comparison with baseline values. Montelukast pretreatment significantly ($P < .001$) increased the bradykinin PC_{20} value (0.85 μg/mL [geometric mean + SE = 1.18; geometric mean − SE = 0.62]) in comparison with placebo (0.18 μg/mL [geometric mean + SE = 0.23; geometric mean − SE = 0.14], Fig 1) and produced a shift of the

bradykinin concentration-response curve to the right. Compared with placebo, montelukast did not have a significant protective effect against methacholine challenge.

When reported as a concentration ratio, montelukast afforded a 4.72-fold protection against bradykinin-induced bronchoconstriction.

In the absence of any drug treatment, baseline urinary LTE_4 excretion was 4.4 ± 0.9 ng/mmol creatinine and increased to 6.3 ± 1.0 ng/mmol after bradykinin challenge but not after methacholine challenge (3.9 ± 0.8 ng/mmol). However, this increase did not reach statistical significance. Similarly, after the administration of montelukast or placebo, there was an increase in urinary LTE_4 excretion after bradykinin challenge (6.6 ± 1.9 and 6.1 ± 1.7 ng/mmol, respectively) compared with the correspondent baseline excretion (3.4 ± 0.9 and 4.3 ± 0.7 ng/mmol, respectively), but once again, the increased urinary excretion of LTE_4 observed after bradykinin challenges never reached statistical significance. Urinary LTE_4 values after methacholine challenges after administration of montelukast and placebo were 3.8 ± 1.2 and 3.1 ± 2.4 ng/mmol, respectively, and did not show any significant changes compared with respective baseline values (3.4 ± 0.9 and 4.3 ± 0.7 ng/mmol, respectively).

In this study we have shown that the pretreatment of asthmatic subjects with montelukast, a cys-LT receptor antagonist, elicits a selective significant protection against bradykinin-induced bronchoconstriction but not against airway response to methacholine.

In spite of these data, we have not been able to demonstrate a significant increase of urinary LTE_4 levels after bradykinin challenge. Interestingly, bradykinin induced an increase of urinary LTE_4 levels in the urine samples collected 2 hours after bradykinin-induced bronchoconstriction in each phase of the study, but these increases did not reach statistical significance, probably because of a very high variability among samples. We chose this time point because it is reported that after allergen challenge, this time point should ensure a good recovery of urinary leukotrienes,[8] although it is not possible to exclude that we might have missed the peak of LTE_4 urinary excretion. Because *in vivo* evidence has shown that the bronchoconstriction induced by cys-LTs is not necessarily followed by an increase of urinary LTE_4 excretion, it is also conceivable to hypothesize that after bradykinin inhalation, the local level of cys-LTs generated might be sufficient to determine a functionally relevant effect but too low or transient to change significantly the whole body production reflected by urinary LTE_4 excretion.

Furthermore, it is not possible to exclude that bradykinin could also upregulate the signal transduction pathways of leukotriene receptors rather than increasing leukotriene release, and this would explain the lack of increase of urinary LTE_4 after bradykinin challenge.

Nevertheless, the observed capacity of montelukast to provide significant protection against bradykinin-induced bronchoconstriction is of great interest, considering that a local activation of the bradykinin-forming cascade is thought to occur in airway responses to a wide range of

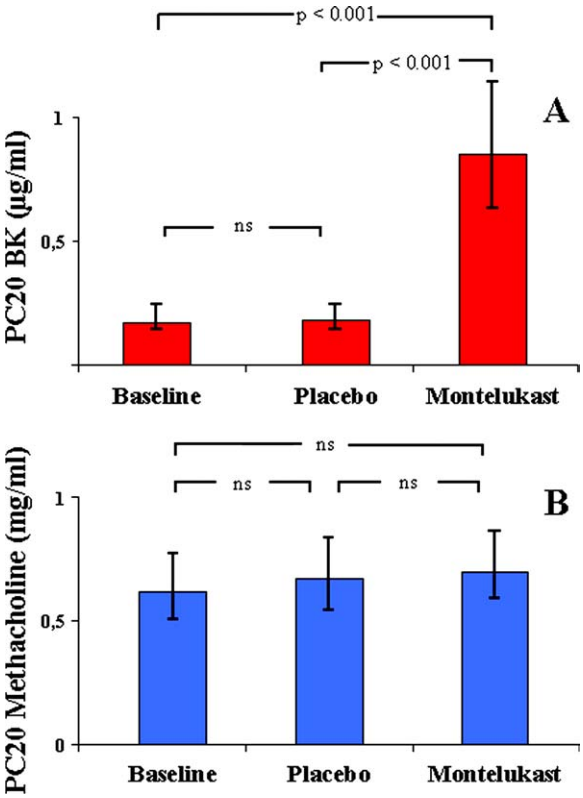

FIG 1. Effect of montelukast and placebo on bradykinin-induced **(A)** and methacholine-induced **(B)** bronchoconstriction in comparison with baseline values in asthmatic subjects. Results are expressed as means ± SE. *BK,* Bradykinin.

inflammatory stimuli, including gastric esophageal reflux, virus infections, and pathophysiologic conditions, such as neurogenic inflammation and excessive activation of lung afferent nerve endings. Therefore those subsets of asthmatic subjects, in which an increased endogenous release of bradykinin is supposed, could represent patients who are likely to obtain the greatest clinical benefit from the use of antileukotriene drugs. In this regard, the described antitussive effects of leukotriene receptor antagonists in patients with cough-variant asthma[9] and respiratory syncytial virus postbronchiolitis[10] could in part be explained by hypothesizing an excessive endogenous bradykinin synthesis and a consequent increased leukotriene generation.

Although it is not possible to provide a conclusive explanation for the mechanism by which the cys-LT pathway is activated in response to inhaled bradykinin, this is the first study, to our knowledge, that shows in human subjects that montelukast, a selective cys-LT antagonist, significantly reduces the airway response to bradykinin. This lends further support to the hypothesis that bradykinin might elicit bronchoconstriction indirectly through the release of cys-LTs. We believe that these findings, considering the importance of bradykinin as a local mediator involved in a wide range of airway responses to offending stimuli, might have clinical relevance, thus providing further explanation for the

anti-inflammatory activity and therapeutic utility of leukotriene receptor antagonists.

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Determination of IgG subclasses: A need for standardization

To the Editor:

Measurement of IgG subclasses is widely performed as part of the laboratory evaluation of immunologic deficiencies. In clinical laboratories, IgG subclass measurements are generally performed by automated nephelometry. There is, however, no international reference preparation, and the 2 major providers of IgG subclass kits use different calibration strategies. In the Sanguin (Amsterdam, The Netherlands) assay (PeliClass human IgG subclass kit), the IgG subclass levels in the calibrator are derived from the World Health Organization 67/97 reference preparation (using IgG subclass values ascertained by Klein et al[1]), whereas in the assay from The Binding Site (TBS; Birmingham, United Kingdom), IgG subclass levels in the calibrator are determined from the Certified Reference Material 470 (CRM 470) (using IgG subclass values ascribed to Schauer et al[2]). The latter is the Reference Preparation for Proteins in Human Serum, released from the College of American Pathologists and the Bureau Communitaire de Référence.

The concentration of IgG subclasses is strongly age-dependent, and most clinical laboratories rely on reference ranges made available by the manufacturer. The reference ranges provided by Sanguin were obtained by compiling several studies.[3] In all of the studies, the World Health Organization 67/97 standard was used, and the majority of the studies applied radial immunodiffusion. The reference ranges provided by TBS were established with 312 healthy children by nephelometry using CRM 470 as calibrator.[2]

We evaluated whether Sanguin and TBS give comparable results and whether interpretation of the results is identical. IgG subclasses were determined in 94 children with increased susceptibility for respiratory tract infection. Each of the children had at least 5 episodes of upper respiratory tract infections complicated by otitis media or chronic (longer than 3 weeks' duration) draining ears and/or had at least 3 lower respiratory tract infections with radiographic evidence of pneumonia in at least 2 of these episodes. The age distribution was as follows: 8, 30, 17, 10, 20, 6, and 3 for 2, 3, 4, 5, 6-10, 11-15, and 16-23 years, respectively (50 boys, 44 girls). All assays were performed on an Immage nephelometer (Beckman-Coulter, Brea, Calif). The Wilcoxon signed rank test was used for statistical comparison. The precision was determined by analyzing 2 aliquots of a serum pool on 20 different days.[4]

For Sanguin and TBS, respectively, total precision values were 3.19% and 5.05% for IgG1, 3.84% and 3.62% for IgG2, 2.38% and 6.24% for IgG3, and 6.42% and 4.50% for IgG4; within-run precision values were 2.45% and 3.1% for IgG1, 3.32% and 2.49% for IgG2, 2.4% and 4.7% for IgG3, and 3.94% and 4.18% for IgG4; between-day precision values were 2.05% and 3.99% for IgG1, 1.93% and 2.63% for IgG2, 0 and 4.1% for IgG3, and 5.07% and 1.66% for IgG4.

The results of the comparison are presented in Fig 1. For IgG1, the respective median (95% CI) values for Sanguin and TBS were 5.84 g/L (5.45 to 6.69) and 6.045 g/L (5.43 to 6.65) ($P = .1037$). For IgG2, the respective values were 1.1 g/L (0.96 to 1.27) and 1.1 g/L (0.95 to 1.33) ($P = .5943$). For IgG3, significantly higher values ($P < .0001$) were found with TBS (median value, 0.508 g/L [95% CI: 0.425 to 0.554]) than with Sanguin (median value, 0.315 g/L [95% CI: 0.266 to 0.36]). For IgG4, significantly lower values ($P < .0001$) were found with TBS (median value, 0.143 g/L [95% CI: 0.104 to 0.193]) than with Sanguin (median value, 0.2 g/L [95% CI: 0.16 to 0.25]). For IgG4, only 71 samples were included in the statistical analysis because many samples had IgG4 values