

Bronchial responsiveness to methacholine is increased in citrus red mite (*Panonychus citri*)-sensitive children without asthmatic symptoms

K.-U. MIN^{*†}, Y.-K. KIM^{*†}, H.-S. PARK[‡], M.-H. LEE[§], B.-J. LEE^{*†}, J.-W. SON^{*†}, Y.-Y. KIM^{*†} and S.-H. CHO^{*†}

**Department of Internal Medicine, Seoul National University College of Medicine, Seoul, †Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul; ‡Department of Allergy and Clinical Immunology, Ajou University School of Medicine, Suwon; §Department of Paediatrics, Cheju University College of Medicine, Cheju, Korea*

Summary

Background A recent investigation has suggested that citrus red mite (*Panonychus citri*, CRM) is a common sensitizing allergen among children living around citrus farms.

Objective A cross-sectional survey was performed to evaluate the bronchial responsiveness to methacholine in CRM-sensitive children without asthmatic symptoms.

Subjects and methods A total of 375 children living around citrus farms were enrolled in this study. There were 201 boys and 174 girls with a mean age of 12.5 (range 7–16) years. Each subject was evaluated by a questionnaire, a skin prick test with 13 common inhalant allergens including CRM, and a methacholine bronchial provocation test (MBPT).

Results Positive rate of MBPT (PC₂₀-methacholine <25 mg/mL) was higher in atopic rhinitic and atopic control children than in nonatopic rhinitic and nonatopic control subjects (41.3% and 33.3% vs 17.2% and 15.4%, respectively, both $P < 0.05$). Slope of dose–response curve (DRS, %/mg/mL) was higher in atopic rhinitic children than in nonatopic rhinitic, atopic control and nonatopic control subjects (14.3 ± 0.87 vs 9.4 ± 0.72 , 10.0 ± 1.37 and 9.2 ± 1.51 , $P < 0.05$, respectively). Both the positive rate of MBPT and the DRS were increased in children with positive skin response to CRM than in those without sensitization (48.2% vs 22.9%, $P = 0.0001$; 15.6 ± 1.26 vs 10.2 ± 0.65 , $P = 0.0001$).

Conclusion Bronchial responsiveness is increased in citrus red mite-sensitive children even if they have no asthmatic symptoms.

Keywords: citrus red mite, bronchial responsiveness, asthma, sensitization, children.

Clinical and Experimental Allergy, Vol. 30, pp. 1129–1134. Submitted 18 May 1999; revised 8 November 1999; accepted 27 December 1999.

Introduction

Bronchial hyperresponsiveness (BHR) to pharmacological agonists such as methacholine or histamine is a characteristic feature of subjects with asthma [1,2]. Those with allergic rhinitis but no history of asthma, however, often show heightened bronchial responsiveness [3–5]. A variety of studies in both children and adults have demonstrated that

bronchial hyperresponsiveness precedes and predicts the development of asthma [6,7].

House dust mites are known as a major cause of respiratory allergy in subtropical areas including Korea [8]. A recent survey revealed that phytophagous mites, particularly citrus red mite (*Panonychus citri*, CRM) was the most important allergen in citrus-cultivating farmers with asthma and/or allergic rhinitis, and that the latent period of CRM-induced asthma was longer than 10 years [9,10]. CRM was also a very common sensitizing allergen in children living around citrus farms, but the prevalence of

Correspondence: S.-H. Cho, Department of Internal Medicine, Seoul National University College of Medicine, 28 Yungun-dong Chongno-gu, Seoul 110–744 Korea.

asthma was not different between the children with sensitization to CRM and those without it [11].

The aim of this study was to determine bronchial responsiveness in relation to allergic rhinitis and atopic status, and to evaluate bronchial responsiveness in CRM-sensitive nonasthmatic children.

Subjects and methods

Subjects

A total of 375 children with a mean age of 12.5 (range 7–16) years, who had been living around citrus farms, were enrolled in this study; there were 174 girls and 201 boys. According to rhinitic symptoms and atopic status, they consisted of 121 atopic rhinitic subjects with a mean age of 12.7 ± 0.20 years, 157 nonatopic rhinitic subjects with a mean age of 12.8 ± 0.19 years, 45 atopic controls with a mean age of 11.8 ± 0.36 years, and 52 nonatopic controls with a mean age of 11.9 ± 0.33 years. Each subject responded to a questionnaire, and underwent a skin prick test to 13 inhalant allergens including CRM, and a methacholine bronchial provocation test (MBPT). The parents gave written informed consent for the study, and the children agreed to participate with parental consent. The study protocol was approved by the Ethics Committee of Seoul National University Hospital.

Methods

A questionnaire developed by International Study of Asthma and Allergy in Children (ISAAC) [12] was translated into Korean, and included demographic data and medical history such as asthmatic and nasal symptoms. Asthmatic symptoms included wheezing during the last 12 months, and nasal symptoms included sneezing or nasal obstruction during the last 12 months. If subjects had experienced recurrent sneezing or nasal obstruction without respiratory tract infections during the last 12 months on the questionnaire, chronic rhinitis was considered to be positive in this respect. If a subject had ever experienced wheezing on the questionnaire, the subject was excluded from this study.

Skin prick tests were performed with a panel of 11 inhalant allergens (Allergopharma Co., Germany), Japanese cedar pollen and CRM. None of the subjects had received antihistamines orally for 3 days preceding the study. The commercially available panel included *Dermatophagoides pteronyssinus*, *D. farinae*, cat fur, molds (*Alternaria* spp. and *Aspergillus* spp.), pollens (tree pollen mixture 1 & 2, grass pollen mixture, mugwort, and ragweed), and cockroach. A positive control of histamine (1 mg/mL, Allergopharma Co., Germany) along with a negative control of diluent (Allergopharma Co., Germany) was included in all tests. CRM and Japanese cedar pollen extracts were prepared as

previously described [9]. Briefly, CRM and Japanese cedar pollen were mixed with modified Coca's solution in 1:20 w/v ratio. After incubation at room temperature for 24 h, it was centrifuged at 3000g for 30 min. The supernatant was dialysed in phosphate-buffered saline at 4 °C for 48 h, and passed through a 0.45- μ m pore for sterilization and then lyophilized. Same amount of glycerin was added with final concentration of 1:40 w/v, and it was used for skin tests. Fifteen minutes after a prick, mean diameter of a weal formed by the allergen was compared with that formed by histamine. If the former was the same or larger than the latter (A/H ratio ≥ 1.0), the reaction was deemed to be positive. Atopy was defined as positive skin response to one or more allergens. If subjects with chronic rhinitis had atopy, atopic rhinitis was considered to be positive in this respect.

Forced expiratory volume in 1 second (FEV₁) was measured with a portable spirometer (Micro Spirometer, Micro Medical, Rochester, Kent, UK), and the largest value of triplicate FEV₁ was adopted. MBPT was carried out using the modified method described by Chai *et al.* [13]. Concentrations of 1.25, 2.5, 6.25, 12.5, and 25 mg/mL of methacholine were prepared by dilution with buffered saline. A Rosenthal-French dosimeter (Laboratory for Applied Immunology, Baltimore, MD, USA) was used to deliver the aerosol generated by a DeVilbiss 646 nebulizer. Subjects inhaled five inspiratory capacity breaths of increasing concentrations of methacholine until FEV₁ fell by more than 20% of its baseline value or the highest concentration was reached. The largest value of triplicate FEV₁ at 90 or 180 s after each inhalation was adopted for analysis. If the concentration of methacholine which caused a 20% fall in FEV₁ (PC₂₀-Methacholine) was less than 25 mg/mL, a subject was considered to be positive in MBPT [14]. The slope of the dose-response curve (DRS), defined as the percentage fall in FEV₁ at the last dose divided by the log of the last concentration of methacholine (mg/mL), was calculated for a continuous index of bronchial responsiveness to methacholine [15].

Statistical analysis

Results were expressed as mean $\pm$ standard error unless otherwise indicated. The chi-square tests, analysis of variance (ANOVA) with Student's *t*-tests, and regression analysis were applied to evaluate the statistical significance. A *P*-value of 0.05 or less was regarded as significant.

Results

Skin response and bronchial responsiveness in the study subjects

Figure 1 shows skin responses to 13 inhalant allergens in the atopic rhinitic and atopic control subjects. *D. pteronyssinus*

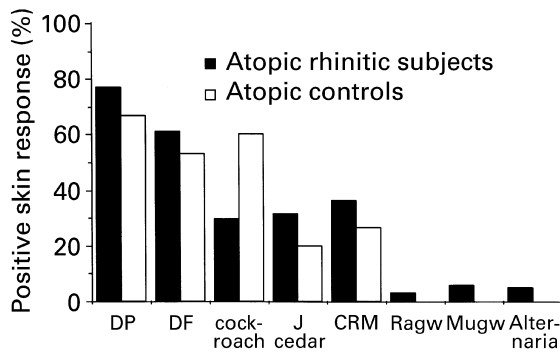


Fig. 1. Positive skin responses to common sensitizing allergens in atopic rhinitic and control subjects. DP: *Dermatophagoides pteronyssinus*; DF: *Dermatophagoides farinae*; J cedar: Japanese cedar pollen; CRM: citrus red mite; Ragw: ragweed pollen; Mugw: mugwort pollen.

was the most common sensitizing allergen among atopic rhinitic and atopic control subjects (76.9% and 66.7%), and followed by *D. farinae* (61.2% and 53.3%), CRM (36.4% and 26.7%), Japanese cedar pollen (31.4% and 20%) and cockroach (29.8%, and 60%).

Table 1 shows positive rate of MBPT in the study subjects. In a cut-off point of PC₂₀-methacholine < 25 mg/mL, the positive rate of MBPT was higher in atopic rhinitic and atopic control children than in nonatopic rhinitic and nonatopic control subjects (41.3% and 33.3% vs 17.2% and 15.4%, respectively, both $P < 0.05$). Figure 2 shows DRS (%/mg/mL) of MBPT in the study subjects. Value of DRS was higher in atopic rhinitic children than in nonatopic rhinitic, atopic control and nonatopic control subjects (14.3 ± 0.87 vs 9.4 ± 0.72 , 10.0 ± 1.37 and 9.2 ± 1.51 , $P < 0.05$, respectively).

Risk factors in relation to bronchial responsiveness in nonasthmatic children

Table 2 shows bronchial responsiveness according to sex,

Table 1. Positive rate of methacholine bronchial provocation test in the study subjects

	AR	NAR	AC	NAC
Number	121	157	45	52
Positive rate	50 (41.3)	20 (17.2)	15 (33.3)	8 (15.4)

AR: atopic rhinitic subjects; NAR: nonatopic rhinitic subjects; AC: atopic controls; NC: nonatopic controls. * $P < 0.05$ compared to that of NAR, AC and NC. PC₂₀-methacholine < 25 mg/mL, number (%).

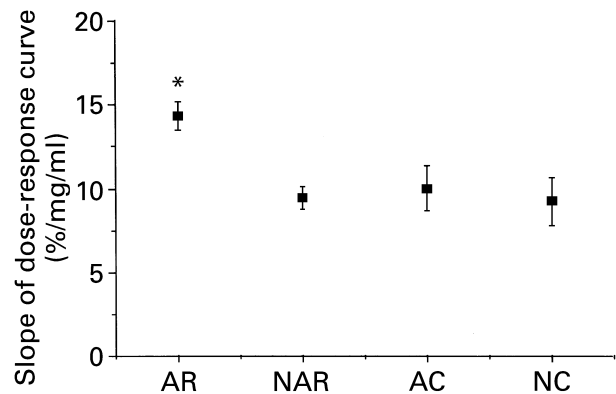


Fig. 2. Slope of dose-response curve in the study subjects. AR: atopic rhinitic subjects; NAR: nonatopic rhinitic subjects; AC: atopic controls; NC: nonatopic controls. Data were expressed as mean $\pm$ SEM * $P < 0.05$ compared to that of NAR, AC and NC.

chronic rhinitis, atopic rhinitis, and skin responses to common sensitizing allergens. There was no significant difference in positive bronchial response to methacholine and in DRS between boys and girls, and between children with positive and negative nasal symptoms. The positive rate to MBPT was higher in atopic children than in nonatopic ones (39.2% vs 16.7%, $P < 0.05$). The DRS (%/mg/mL) was also higher in atopic ones than in nonatopic ones (13.2 ± 0.75 vs 9.3 ± 0.65 , $P < 0.05$). According to skin response to CRM, the positive rate of MBPT was higher in children sensitized to CRM than in those without sensitization (48.2% vs 22.9%, $P = 0.0001$). This trend was similar for the DRS (15.6 ± 1.26 vs 10.2 ± 0.53 , $P = 0.0001$).

Skin indices, defined as ratio of mean weal formed by allergen divided by histamine, to *D. pteronyssinus*, *D. farinae*, Japanese cedar pollen and CRM were positively associated with the DRS ($r = 0.19$, $P = 0.0002$; $r = 0.24$, $P = 0.0001$; $r = 0.19$, $P = 0.0003$; $r = 0.21$, $P = 0.0001$). However, there was no significant association between the skin index to cockroach and the DRS ($r = 0.08$, $P > 0.05$). Among the children with sensitization to *D. pteronyssinus* and *D. farinae*, the DRS (%/mg/mL) was higher in children with chronic rhinitis than in those without it (15.2 ± 1.01 vs 8.7 ± 1.56 , $P < 0.01$; 16.7 ± 1.21 vs 11.8 ± 2.22 , $P < 0.05$, respectively). As for CRM-sensitive children, however, this difference was less marked (12.8 ± 1.02 vs 10.7 ± 2.33 , $P > 0.05$).

Discussion

The results of this study demonstrated that among children without asthmatic symptoms, the positive rate of methacholine bronchial provocation test (MBPT) and the slope of the dose-response curve (DRS) were higher in children

Table 2. Risk factors associated with bronchial responsiveness

	MBPT, %*	P-value	DRS†	P-value
Sex				
Boys (<i>n</i> = 221)	26.5	> 0.05	11.2 ± 0.74	> 0.050
Girls (<i>n</i> = 174)	27.8		10.9 ± 0.67	
Chronic rhinitis				
Positive (<i>n</i> = 278)	27.7	> 0.05	11.5 ± 0.57	> 0.05
Negative (<i>n</i> = 97)	23.7		9.6 ± 1.02	
Atopy				
Positive (<i>n</i> = 166)	39.2	0.0001	13.2 ± 0.75	0.0001
Negative (<i>n</i> = 209)	16.7		9.3 ± 0.65	
Atopic rhinitis				
Positive (<i>n</i> = 121)	41.3	0.0001	14.4 ± 0.87	0.0001
Negative (<i>n</i> = 254)	19.7		9.4 ± 0.59	
<i>D. pteronyssinus</i>				
Positive (<i>n</i> = 123)	43.1	0.0001	14.0 ± 0.90	0.0001
Negative (<i>n</i> = 252)	18.7		9.6 ± 0.58	
<i>D. farinae</i>				
Positive (<i>n</i> = 98)	50	0.0001	15.5 ± 1.08	0.0001
Negative (<i>n</i> = 277)	18.4		9.4 ± 0.53	
Cockroach				
Positive (<i>n</i> = 63)	34.9	> 0.05	12.1 ± 0.03	> 0.05
Negative (<i>n</i> = 312)	25		10.8 ± 0.52	
Japanese cedar pollen				
Positive (<i>n</i> = 47)	57.4	0.0001	16.2 ± 1.46	0.0001
Negative (<i>n</i> = 328)	22.3		10.3 ± 0.52	
Citrus red mite				
Positive (<i>n</i> = 56)	48.2	0.0001	15.6 ± 1.26	0.0001
Negative (<i>n</i> = 319)	22.9		10.2 ± 0.53	

Chronic rhinitis: subjects with recurrent sneezing or nasal obstruction without respiratory tract infections during the last 12 months on a questionnaire; atopy: subjects with positive skin responses (A/H ratio ≥ 1.0) to one or more allergens; atopic rhinitis: subjects with chronic rhinitis and atopy. *PC₂₀-methacholine < 25 mg/mL; †Slope of dose-response curve, %/mg/mL.

with positive skin response to citrus red mite (CRM) than in those without it.

A decrease of FEV₁ greater than 20% is generally accepted as a discriminating end point to separate the normal and hyperresponsive groups. Other methods of expressing the response to bronchial challenge include the DRS [15], and the bronchial responsiveness index (BRindex), defined as the log of the slope of the percentage decline from baseline FEV₁ after the last dose per unit dose of methacholine [16]. These latter two methods may be more useful in epidemiological studies because a value that related well to symptom history can be calculated for the entire sample, even if the subject does not experience a 20% drop in FEV₁. In this study, the DRS as well as the positive rate of MBPT were used to evaluate the bronchial responsiveness.

Actual BHR may be the resultant of actual, variable airway inflammation on top of baseline bronchial responsiveness

[17]. A number of studies suggest that genetic factors might control this baseline hyperresponsiveness [18,19]. However, the largest part of BHR is probably acquired and related to airway inflammation [17], which is also a common feature of allergic rhinitis [20,21]. The present study also showed that bronchial responsiveness was increased in children with atopic rhinitis than in nonatopic rhinitic, atopic control and nonatopic control subjects. It is not clear, however, whether BHR in atopic rhinitis is caused by nasal inflammation resulting in postnasal drip of inflammatory mediators which directly alters bronchial smooth muscle contractility, or airway inflammation itself [22].

A variety of studies in both children and adults have demonstrated that bronchial responsiveness precedes and predicts the development of asthma [6,7]. One of the more interesting studies was a case-cohort study performed by

Zong *et al.* [6], in which 80 subjects with BHR but no asthmatic symptoms were matched with 80 controls, and followed up 2 years later with regard to their asthma risks. Subjects with increased bronchial responsiveness had a four- to fivefold greater risk of developing asthma in the follow-up interval when compared to subjects without increased bronchial responsiveness.

In our previous study [11], CRM was a common sensitizing allergen in children living around citrus farms. However, there was no difference in the prevalence of asthma between the children with and without sensitization to CRM, although the prevalence was higher among the HDM-sensitive children than among those without it. In farmers with CRM-induced asthma, the latent period from initial exposure to onset of asthmatic symptoms was longer than 10 years [9,10]. Our present study showed that among children living around citrus farms, the bronchial responsiveness was increased in those with sensitization to CRM than in those without sensitization, irrespective of nasal symptoms. This finding suggests that asymptomatic CRM-sensitive children may develop symptomatic asthma beyond childhood, if they will be further exposed to CRM-derived allergens.

In conclusion, this study shows that bronchial responsiveness is increased in CRM-sensitive children even if they have no asthmatic symptoms.

Acknowledgement

This study was supported by a research grant (HMP-98-M-1-0001) from the Ministry of Health and Welfare, Republic of Korea.

References

- Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981; 68:347–55.
- Battemore PK, Asher MI, Harrison AC, Mitchell EA, Rea HH, Stewart AW. The interrelationship among bronchial hyperresponsiveness, the diagnosis of asthma and asthma symptoms. *Am Rev Respir Dis* 1990; 142:549–54.
- Eggleston PA. Upper airway inflammatory diseases and bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1988; 81:1036–41.
- Ramsdale EH, Morris MM, Roberts RS, Hargreave FE. Asymptomatic bronchial hyperresponsiveness in rhinitis. *J Allergy Clin Immunol* 1985; 75:573–7.
- Braman SS, Barrows AA, DeCotiis BA, Settipane GA, Corrao WM. Airway hyperresponsiveness in allergic rhinitis. *Chest* 1987; 91:671–4.
- Zhong NS, Chen RC, Yang MO. Is asymptomatic bronchial hyperresponsiveness an indication of potential asthma? A two-year follow-up of young students with bronchial hyperresponsiveness. *Chest* 1992; 102:1104–9.
- Hopp RJ, Townley RG, Biven R, Bewtra AK, Nair NM. The presence of airway reactivity before the development of asthma. *Am Rev Respir Dis* 1990; 141:2–8.
- Kim YY, Cho SH, Kim WK, Park JK, Song SH, Kim YK, Jee YK, Ha MN, Ahn YO, Lee SI, Min KU. Prevalence of childhood asthma based on questionnaires and methacholine bronchial provocation test in Korea. *Clin Exp Allergy* 1997; 27:761–8.
- Kim YK, Son JW, Kim HY, Park HS, Lee MH, Cho SH *et al.* New occupational allergen in citrus farmers: citrus red mite (*Panonychus citri*). *Ann Allergy Asthma Immunol* 1999; 82:223–8.
- Kim YK, Son JW, Kim HY, Park HS, Lee MH, Cho SH *et al.* Citrus red mite (*Panonychus citri*) is the most common sensitizing allergen in citrus farmers with asthma and rhinitis. *Clin Exp Allergy*, 1999; in press.
- Lee MH, Kim YK, Min KU, Lee BJ, Cho SH, Park HS, Kim YY. Citrus red mite (*Panonychus citri*) is a common sensitizing allergen in children living around citrus farms in Korea. *Annals Allergy Asthma Immunol*, in press.
- Pearce N, Weiland S, Keil U, Langridge P, Anderson HR, Strachan D, Bauman A, Young L, Gluyas P, Ruffin D *et al.* Self-reported prevalence of asthma symptoms in children in Australia, England, Germany and New Zealand: an international comparison using the ISAAC protocol. *Eur Respir J* 1993; 6:1455–61.
- Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR *et al.* Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56:534–9.
- Kim YK, Son JW, Lee SR, Kim WK, Cho SH, Lee MH, Koh YY, Min KU, Kim YY. Bronchial responsiveness to methacholine in general population without bronchial asthma. *J Asthma Allergy*, 1998; 18:416–25.
- Peat JK, Salome CM, Berry G, Woolcock AJ. Relation of dose–response slope to respiratory symptoms in a population of Australian school children. *Am Rev Respir Dis* 1991; 144:663–7.
- Burrows B, Sears MR, Flannery EM, Herbison GP, Holdaway MD. Relationships of bronchial responsiveness assessed by methacholine to serum IgE, lung function, symptoms and diagnoses in 11-year-old New Zealand children. *J Allergy Clin Immunol* 1992; 90:376–85.
- Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P *et al.* Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- Koh YY, Lee MH, Kim CK, Min YG, Kim YK, Min KU, Kim YY. A familial predisposition in bronchial hyperresponsiveness among patients with allergic rhinitis. *J Allergy Clin Immunol* 1998; 102:921–6.
- Hopp RJ, Bewtra AK, Biven R, Nair NM, Townley RG. Bronchial reactivity pattern in nonasthmatic parents of asthmatics. *Ann Allergy* 1988; 61:184–6.

- 20 Boulet LP, Turcotte H, Laprise C, Lavertu C, Bedard PM, Lavoie A, Hebert J. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 1997; 27:52–9.
- 21 Stevens WJ, Vermeire PA. Bronchial responsiveness to histamine and allergen in patients with asthma, rhinitis, cough. *Eur J Respir Dis* 1980; 61:203–12.
- 22 Naclerio RM, Proud D, Togias AG, Adkinson NF Jr, Meyers DA, Kagey-Sobotka A, Plaut M, Norman PS, Lichtenstein LM. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985; 313:65–70.