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The Study of Bronchial Hyperresponsiveness in Asthmatic Children by Forced Oscillation Technique

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SUMMARY **We have studied the bronchial hyperresponsiveness (BHR) of children with normal controls and asthma by methacholine inhalation challenge, using a forced oscillation method. Four parameters, respiratory conductance (Grs), bronchial responsiveness ($PD_{35}Grs$), bronchial sensitivity (Dmin) and reactivity (SGrs) were studied. There were three patterns of dose-response curves identified in this study, which were signifciantly correlated to the clinical severity of asthma. ($r = 0.846$, $p < 0.001$, Spearman's rank correlation). There were significant negative correlations between control Rrs (Rrs cont.) and age ($r = 0.514$, $p < 0.001$) or body height ($r = 0.685$, $p < 0.001$). Positive correlations between SGrs and subjects' age ($r = 0.457$, $p < 0.001$) and body height ($r = 0.496$, $p < 0.001$) were also noted. In the normal controls, Dmin and $PD_{35}Grs$ were over 25 units and 50 units, respectively. The Grs for normal children was statistically higher than that of asthmatic children ($p < 0.05$). In the asthmatic children, there were significant differences among all subgroups in $PD_{35}Grs$ ($p < 0.001$) and Dmin ($p < 0.01$). In summary, the bronchial provocation test using the forced oscillation technique is simple, fast and easy to be applied to children. In addition to being capable of investigating BHR, it may offer valuable information for the clinical diagnosis and treatment of asthmatic children.**

Bronchial hyperresponsiveness (BHR) is an essential etiological factor in bronchial asthma.[1] To evaluate individual BHR, Chai *et al.* have reported a standardized procedure for bronchial inhalation challenge.[2] Their method is used to assess BHR employs FEV1 (forced expiratory volume in 1 second) as the major parameter and requires patients to perform a forced flow volume test repeatedly. It is thus a time-consuming procedure and cannot be easily applied to children, because young subjects are not able to perform forced expiratory maneuver or the panting technique many times after every inhalation. Moreover, forced expiration can itself induce bronchoconstriction.[3] In 1970, Hyatt *et al.* demonstrated that total respiratory resistance (Rrs) could be measured with an oscillation technique.[4] Using this principle, Takishima *et al.*[5] attempted to improve the bronchial provocation test by using a sine-wave pressure generator and a loudspeaker box system to measure the continuous changes of Rrs with minimum cooperation of the subjects. Moreover, bronchial sensitivity and reactivity (Dmin and SGrs, respectively) can be measured at the same time. This method has been recognized as an alternative method for carrying out bronchial provocation test. In the pediatric field, it would be interesting to detect the BHR of asthmatic children by this method and evaluate its relationship to clinical severity of asthma. In the present study, we have evaluated the BHR in asthmatic children age 5 to 16 years with various clinical severity, and assessed the basal differences among them.

PATIENTS AND METHODS

Subjects

One hundred and seventeen asthmatic children and thirteen normal children without respiratory disorders were the subjects of this study (Table 1). The clinical diagnosis of bronchial

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Table 1. Anthropomorphic data.

	Asthma			Normal
	Mild	Moderate	Severe	
Number	36	50	31	13
Age (yrs)	9.8 ± 2.9*	9.4 ± 2.3	9.7 ± 2.2	10.1 ± 2.2
Sex (M:F	14:12	31:19	16:15	8:5
Height	136.8 ± 16.1	133.2 ± 121.1	132.8 ± 11.2	135.0 ± 13.1
IgE (IU)	1222.1 ± 132	998.0 ± 211.9	1365.2 ± 123.7	433.2 ± 221.2**
FEV_1 (%)	64.1 ± 3.9	70.3 ± 8.5	53.1 ± 4.4	89.1 ± 12.3

FEV_1 = forced expiratory volume in one second.
*Mean ± S.D.
**Significantly lower than the asthmatic children ($p < 0.05$, Student's t test).

asthma was based on a characteristic history of recurrent attacks of dyspnea with perceptible wheezing and, after more than a year of follow-up, the diagnosis was established. All asthmatic children reacted to the causative allergens by a skin test and the allergic state of each patient had been analyzed by IgE-PRIST (paper radioimmunosorbent test) and IgE-RAST (radioallergosorbent test) with house dust mite, *Dermatophagoides pterosynosa* (D.p.), which is one of the major allergens in Taiwan. The patients were asymptomatic at the time of the study and had not received any medication for at least 8-96 hr prior to it, according to the standard inhalation procedure.[2] They were divided into three subgroups according to clinical severity (minimal, moderate, and severe).[6] The normal control children had no respiratory disease and no personal or family history of allergic disease.

Informed consent was obtained from the patient's guardians or their parents before the examination.

Methods

Bronchial provocation tests were carried out with an astograph (TCK-6100, CHEST, Japan), which housed 12 nebulizers. Nebulizers No. 2-11 contained 3 ml of methacholine chloride solution (Daichi pure chemicals, Co., Ltd., Tokyo, Japan) in stepwise increased concentrations, i.e., 0.048, 0.098, 0.19, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, and 25.0 mg/ml, respectively. Nebulizer No. 12 contained 3 ml of 2.5 mg/ml of terbutaline as the bronchodilator for relieving bronchospasm. The nebulizer were driven by a constant air flow of 5 LPM (liter per minute) from the air compressor of the apparatus. The subjects were tested in a seated position with nose clip and were instructed to breath normally. Their cheeks were compressed by a balloon to minimize oral pressure. All examinations were performed between 1 and 4 P.M. to avoid changes due to circadian rhythm of pulmonary function. The nebulizers were then actuated in sequence beginning with No. 1 (one minute for each nebulizer). Respiratory resistance (Rrs) was directly written with an X-Y recorder (Graphtec WX-2400). When Rrs increased to twice the baseline value or patients showed symptoms of intolerance such as difficult breathing or chest tightness, the test was interrupted immediately and terbutaline was inhaled. Nebulization was continued to the last concentration (25.0 mg/ml) of methacholine if there was no apparent change in Rrs.

In respondents (Fig. 1), bronchial sensitivity was defined by the cumulative dose (Dmin) of methacholine required to provoke a positive reaction. Since Dmin is dependent on the flow rate and time of nebulization, it is best expressed in methacholine units. One unit is equal to one minute of inhalation of aerosal solution at 1.0 mg/ml of methacholine during quiet tidal breathing.[2] Respiratory conductance (Grs) was calculated from the reciprocal of Rrs (1/Rrs). Because the slope of Grs (SGrs = Grs/t) in a positive reaction is more linear than that of Rrs, SGrs (in L/sec/cm H_2O/min) is defined as the bronchial reactivity. The bronchial responsiveness was expressed as the cumulative dose of methacholine required to produce a 35% decrease in SGrs (PD_{35}SGrs).

Statistics

Statistical significance of the data between or among the different groups of patients was analyzed with the Student's t test or F test (one way analysis of variance, ANOVA). Simple linear regression analysis was performed to examine the relationship between parameters. Spearman's rank correlation was used to test the correlation between severities of asthma and responsible patterns of bronchial provocation test.

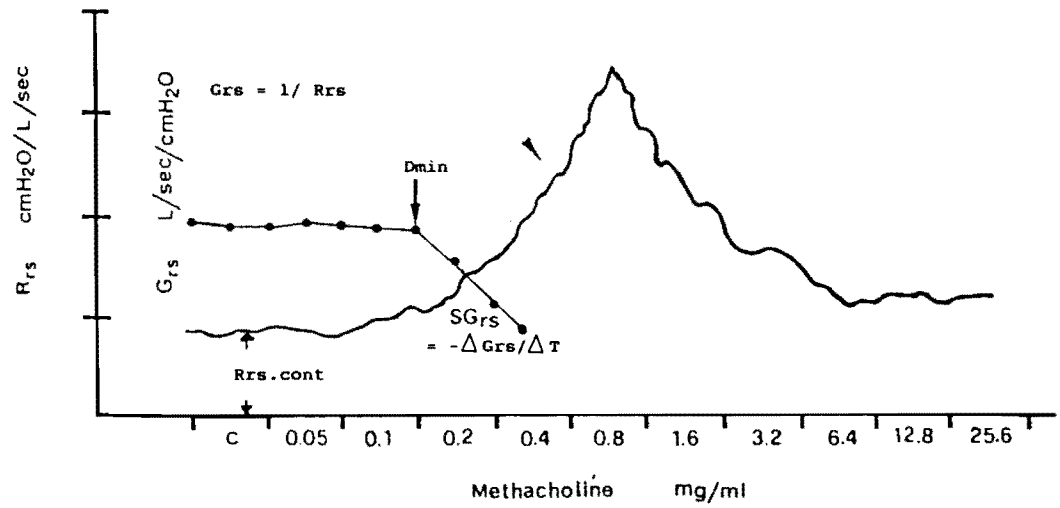

Fig. 1 A typical dose–response curve derived from the methacholine challenge test in an asthmatic children using the oscillation technique. (——): respiratory resistance (Rrs); (.——.): respiratory conductance (Grs). Arrow head indicates the point where terbutaline was inhaled. The X–axis also represents the time course of the test; each scale = 1 minute.

RESULTS

The patterns of methacholine inhalation test.

There were three kinds of dose-response curves identified in this study (Fig. 2). The type I curve was a flat line across the whole test range, resulting from the stable breathing of a normal child. But there were two different results in the subjects that had this kind of curves. The type Ia was his forced expired volume in one second (FEV_1) did not decrease more than 20% after study as compared to the basal level, and the type Ib was the subject produced a greater than 20% drop in FEV_1, though his Rrs did not increase up to the maximum concentration of methacholine hydrochloride of 25 mg/ml. Type II represented the most typical triangular shape for curves of positive reaction. After the Rrs remained at an almost constant value for a short period, it increased curvilinearly at various threshold concentrations of methacholine and decreased rapidly after the inhalation of terbutaline. According to their threshold concen-

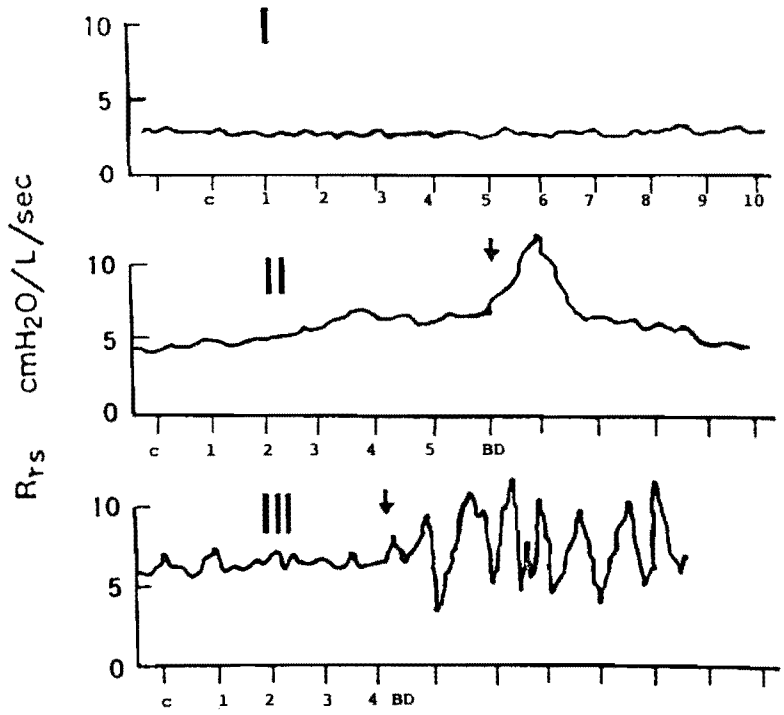

Fig. 2 Typical patterns of Rrs during methacholine inhalation test by oscillation method. Values of methacholine concentration (mg/ml) were as follows: (C) saline; (1) 0.048; (2) 0.098; (3) 0.19; (4) 0.039; (5) 0.78; (6) 1.56; (7) 3.125; (8) 6.23; (9) 12.5; (10 25.0. BD denotes bronchodilator. The arrow shows the point at which wheezine appeared.

tration (Dmin), the type II patients could divided to two groups. Type IIa patients had Dmin greater than 12 mg/ml, and Type IIb patients were below this concentration. The third type was a coughing type, and it was difficult to detect the obvious change in Rrs, although wheezing and dyspnea were present. The relationship between the types of dose-response curves and the clinical severity of asthma are summarized in Table 2. In normal children, approximately 85% belong to the non-responder type, the type Ia. Others had very mild degree of reactive airway response. In asthmatic children, there was a significant correlation between responsive curves and clinical severity ($r = 0.846$, $p < 0.001$, Spearman's rank correlation). The type Ia curves occurred only in about 11.1% and 4% of mild and moderate degree of asthma patients, respectively. On the contrary, type III curve appeared to be the major pattern in moderate and severe types.

The correlation coefficients between various parameters determined by forced osillation methods and patients' age and body height were shown in Table 3. There were good negative correlations between control Rrs (Rrs cont.) and age ($r = 0.514$, $p < 0.001$) or body height ($r = 0.685$, $p < 0.001$). Significant positive correlations between SGrs and subjects' age ($r = 0.457$, $p < 0.001$) and body height ($r = 0.496$, $p < 0.001$) were also noted.

Table 4 shows the comparisons of the respiratory conductance (Grs), bronchialresponsiveness (PD_{35}SGrs), bronchial sensitivity (Dmin), bronchial reactivity (SGrs) among the normals and various severities of asthmatic children. In the normal controls, Dmin and PD_{35}SGrs were over 25 units and 50 units respectively. Grs was 0.24 ± 0.01 L/sec/cmH_2O (mean $\pm$ SD), which being statistically higher than the Grs (0.15 ± 0.05 L/

Table 2. Distribution of the pattern of dose–response curves of methacholine inhalation test.

Clinical Symptom	Curve type					
	Ia	Ib	IIa	IIb	III	Total
Normal	9*	2	2	0	0	13
Mild	3	8	17	8	0	36
Moderate	1	6	16	17	10	50
Severe	0	1	6	13	11	31

*No. of patients.

Table 3. The correlation between various parameters determined by oscillation method and patients' age and body height.

Test	Correlation coefficient (r)	
	Age	Body Height
Rrs (cmH_2O/L/sec)	−0.514*	−0.685*
Grs (L/sec/cmH_2O)	0.314	0.388
SGrs (L/sec/cmH_2O/min)	0.457*	0.496*
Dmin (U.)	0.243	0.102
PD_{35}SGrs (U.)	0.122	0.027

r = Pearson's correlation coefficient.
*$p < 0.001$.

Table 4. The results of methacholine inhalation challenge test by the forced oscillation method.

Test	Asthma				Normal
	Mild	Moderate	Severe	ANOVA*	
Grs (L/sec/cmH_2O)	0.16 ± 0.06	0.14 ± 0.04	0.14 ± 0.01	N.S.	0.24 ± 0.01
SGrs (L/sec/cmH_2O/min)	0.016 ± 0.002	0.014 ± 0.001	0.014 ± 0.001	N.S.	(–)
Dmin (U.)	16.0 ± 1.6	4.9 ± 1.3	1.5 ± 0.5	$P < 0.001$	> 25
PD_{35} SGrs (U.)	35.2 ± 3.4	17.2 ± 2.8	7.6 ± 1.4	$P < 0.01$	> 50

*One way ANOVA test was used to examine the difference among three asthmatic groups.

sec/cmH_2O) ($p < 0.05$, Student's t test) for all asthmatic children. In the three asthma subgroups, there were significant difference between all subgroups in PD_{35}SGrs ($P < 0.001$, one way of ANOVA) and Dmin ($p < 0.01$, ANOVA). No statistical differences were found among all three subgroups in Grs and SGrs.

DISCUSSION

The evaluation of individual bronchial hyperresponsiveness is indispensable not only in asthmatic adult but also in asthmatic children. Use of methacholine inhalation challenge in normal subjects and asthmatics demonstrates that all asthmatics have increased bronchial responsiveness.[7,8] It is time-consuming to perform standardized procedures of methacholine inhalation challenge in younger children. Recently, a convenient device using the forced oscillation method has been available and easy to perform in children with a high reliability and reproducibility.[9,10] In the present study, we performed methacholine inhalation challenge through this method in asthmatic children and found that there was a close relationship between the bornchial sensitivity as clinical severity of asthmatic children (Table 2).

Measurement of Rrs by the forced oscillation method instead of body plethysmography or esophageal balloon is well accepted in children.[11] Mansell *et al.*[12] measured Rrs in 79 normal children ranging in age from 3 to 17 yr and in height from 92 to 192 cm. They found a negative correlation between Rrs and height [Rrs = antilog (1.877-0.0089 x height in cm), r = -0.851. Stanescu and his co-workers[13] reported similar results between Rrs and height in 130 children (Rrs = 24.7-0.13 x height in cm, r = 0.74). In the present study, we have observed a similar correlation between Rrs and height ($p < 0.001$) and age ($p < 0.001$), respectively (Table 3). Rrs measured by forced oscillation method equals airway resistance (Raw) plus tissue resistance, such as that of the lung, the thoracic wall and the liver.[14] Raw accounted for approximately two thirds of Rrs in normal adults,[15] but these values have been found to be almost identical to each other in children.[16]

In the present study, we have found that there were significant differences between the mean values for PD_{35} SGaw and Dmin among the three asthmatic subgroups and related to their clinical severities (Table 4). The exact cause of bronchial hyperresponsiveness in asthma is not certain. Previous studies have reported that BHR is induced by respiratory exposure to ozone, infection, and antigen challenge[17,18] but the increase in reactivity dose not last very long. The BHR of asthma was more stable and persistent.[19] In this study, the more severe degree asthmatic children have high BHR, while in the mild attack patients, it was not so high. We thought that children with asthma established BHR gradually through chronic inflammatory process after repeated allergen challenge,[20] and by the inflammatory mediator released during the late phase reaction of asthma attack.[21]

Our present study indicated that there was no correlation between the bronchial sensitivity (Dmin) and reactivity (SGrs) of all responders to the methacholine test. It is possible that bronchial sensivitity and reactivity are not determined by the same factors. Studies have reported that bronchial reactivity is dependent on the tension of the vagal nerve, and bronchial sensitivity on the tension of the adrenergic nerve.[22,23] Mochizuki *et al.*[18] reported that SGrs directly suggests the degree of bronchoconstriction and reflects the isolated muscle tone. But our findings indicate that SGrs of asthmatic children was not statistically different among the three asthmatic subgroups, nor between the normal controls and asthmatic children. On the other hand, it showed that bronchial sensitivity, Dmin, of normal controls and mild degree of asthmatic children were remarkably higher than that of children with more severe degree asthma. The mechanism of this difference is still unknown and need further study.

In conclusion, the bronchial provocation test using the oscillation technique is simple, fast, reliable and easy to apply to children. In addition to being capable of investigating BHR, it may offer valuable information for the clinical diagnosis and treatment of asthmatic children. It was also demonstrated that there was a close relationship between the level of increased BHR and the clinical severity of asthmatic in children, as is in the case in asthmatic adults.

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