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Standardization of Methacholine Inhalation Challenge by a Reservoir Method

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SUMMARY **Standardization of methacholine inhalation challenge (MIC) by a reservoir method was performed at Respiratory Unit, Chulalongkorn Hospital. One hundred subjects, including 20 non-smoking healthy subjects, 20 patients with isolated chronic cough, 20 patients with isolated allergic rhinitis, 20 patients with stable chronic obstructive bronchitis, and 20 patients with mild bronchial asthma, were scheduled to perform the test. The aerosolized methacholine was produced by an atomized nebulizer of the Provocation test I (Pari-Starnberg) and the aerosol was kept in a reservoir bag. It was inhaled by each subject via a slow vital capacity. Increasing concentrations of methacholine (0, 0.5, 1, 5, 10, and 25 mg/ml were used. None of the healthy subjects had increased bronchial hyperresponsiveness (BHR). Sixty percent of patients with chronic cough, 60% of patients with allergic rhinitis, 95% of patients with chronic obstructive bronchitis, and 100% of patients with asthma were found to be positive in the MIC tests. No serious effect from methacholine during and after the tests was found. It was concluded that MIC can be easily performed by a reservoir with reproducible results to demonstrate BHR.**

Airway inflammation and bronchial hyperresponsiveness (BHR) are important pathogenesis in bronchial asthma.[1] However, other conditions such as allergic bronchitis and chronic obstructive pulmonary disease may also have increased bronchial reactivity.[2,3] The inhalation bronchial challenge test with pharmacologic agents such as histamine or methacholine is performed to demonstrate or exclude bronchial hyperresponsiveness. The challenge test can be performed by several methods.[4] A reservoir method has been recently found to have some advantage of excellent and reproducible results due to the stable deposition of the pharmacologic agents in the bronchial system.[5] There is no information about the standardization of the bronchoprovocative test by this method in Thailand. The present study was designed to evaluate and standardize the methacholine inhalation challenge (MIC) by a reservoir method in Thai subjects.

MATERIALS AND METHODS

Subjects

One hundred subjects were scheduled for the methacholine inhalation challenge by the reservoir method at Respiratory Unit, Chulalongkorn Hospital. There were 20 non-smoker healthy persons (group I), 20 patients with an isolated chronic cough for more than 3 weeks (group II), 20 patients with allergic rhinitis (group III), 20 patients with stable chronic obstructive pulmonary disease (group IV) and 20 patients with mild bronchial asthma (group V). Normal healthy subjects were medical personel and medical student volunteers. They also had no history of atopy such as bronchial asthma, allergic rhinitis or atopic eczema in their families. Many patients with chronic cough were consulted from general medical clinics. Almost all of them had received the diagnosis of upper res-

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piratory tract infection 3-4 weeks earlier, followed by persistent cough for at least 3 weeks. All of them had a dry cough or slightly productive cough with minimal mucoid sputum. There was no special daytime or night-time preference of coughing. They also had no other symptoms such as dyspnea, wheezing, or postnasal drip. Patients with allergic rhinitis (positive skin tests) were selected from the Allergy Clinic. The rhinitis patients with symptoms of asthma were excluded. Patients with chronic obstructive pulmonary disease were selected on the basis of a history of chronic productive sputum for at least 3 months of each of the last 2 years and spirometry showing FEV_1/FVC of less than 70% of that predicted and no improvement in FEV_1 greater than 15% after inhalation of bronchodilator. Patients with asthma were selected from the Chest Clinic and the Allergy Clinic. They were classified as having a mild degree of asthma which was defined as few clinical symptoms (exacerbation of cough and wheezing no more often than 1-2 times/week, nocturnal attack no more than 1-2 times/month) and minimal or no evidence of airway obstruction on spirometry. If the baseline spirometry showed a decrease of FEV_1/FVC of less than 70% of that predicted, they would show an improvement in FEV_1 greater than 15% after inhalation of bronchodilator. All subjects were asked to refrain from using corticosteroids, antihistamines, bronchodilators, caffeine and other drugs at least 12-24 hours before testing.

Procedure

The method of methacholine inhalation challenge by the reservoir bag has been previously described. Briefly, solutions of methacholine in a citrate buffer were prepared under sterile conditions for each concentration: 0 (diluent), 0.5, 1, 5, 10 and 25 mg/ml. All solutions were stored for not more than 3 months at 4°C. The inhalation challenge was performed by the Provocation test I, Number 64.60, (Pari-Starnberg, Germany). The equipment had an atomized nebulizer part filled with 6 ml of methacholine solution to produce the aerosol. The aerosolized methacholine was kept into a 10 l reservoir bag, then was inhaled into the respiratory tract of the subject.

Before methacholine inhalation, baseline spirometric tests were performed with subjects standing using the Autospiror Discom-21 (Chest Corporation, Tokyo, Japan). At least three satisfactory and two reproducible spirometric maneuvers were required according to American Thoracic Society recommendations.[6] The largest FEV_1 value from the acceptable maneuver was used for the baseline FEV_1. Then subjects inhaled diluent aerosol from the reservior via a slow inspiratory vital capacity maneuver until the bag was empty. Three minutes after the inhalation, the spirometry was repeated. The largest FEV_1 from an acceptable maneuver was used as the post-diluent control value. After that, subjects inhaled an increasing concentration of the methacholine aerosol (0.5, 1, 5, 10, and 25 mg/ml, respectively) from the reservoir bag with 10 minutes spacing between doses. Spirometry was performed in a similar manner after inhalation of each concentration of methacholine, the largest FEV_1 from an acceptable maneuver was selected for analysis. If the decline of FEV_1 after any inhalation was more than 20% of the baseline FEV_1, the test was terminated. If the decline of FEV_1 was 15-20%, the same concentration of methacholine was given again to avoid a serious effect and to get the consistent concentration. If the FEV_1 still failed to decline by 20%, the next highest concentration with 5 l of the aerosol volume was given. At the end of the test the subjects who had a decline of FEV_1 of greater than 15% were administered with salbutamol from a metered-dose inhaler via a spacer and spirometry was repeated 10 minutes later. Subjects were told about the possible late phase reaction occurring 6-8 hours after the test and they were discharged from the unit after their FEV_1 had returned to within 10% of their baseline value.

Data analysis

Subjects were categorized as having BHR (positive test) if they showed a more than 20% decrease in FEV_1 (PC_{20}) from baseline after inhalation of diluent or any concentration of methacholine up to and including 25 mg/ml.[7] They were also classified as having pronounced BHR (severe degree or highly positive response) if their FEV_1 declined more than 20% from the baseline value after inhalation of less than 5 mg/ml methacholine.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). Results were presented as the mean ± standard deviation (SD). For comparison of the mean value, the unpaired *t*-test was used. A p value of less than 0.05 was considered statistically significant.

RESULTS

The results of this study are presented in Tables 1, 2 and Fig. 1. The mean value ± the standard deviation for the ages, heights, FVC, %FVC, FEV_1, and $\%FEV_1/FVC$ in the five groups studied are shown in Table 1. There were no statistically significant differences in ages and heights among the subjects in groups I, II, III, and V. There were also no statistically significant differences in the FVC, %FVC, FEV_1, and $\%FEV_1/FVC$ among the subjects in groups I, II, and III. Compared with normal subjects, asthmatic patients had slightly lower values for these spirometric para-

Table 1. Demographic characteristics and lung function parameters of subjects undergoing methacholine challenge testing.

	Normal		Chronic cough		Allergic rhinitis		COPD		Asthma	
	M	F	M	F	M	F	M	F	M	F
1. Age	38 ±13.4	34.5 ±11.3	35.5 ±6.3	32.4 ±6.7	34.2 ±7.1	37.2 ±7.8	64.6 ±4.9	58.2 ±3.9	37.1 ±6.2	39.8 ±8.3
2. Height	167.5 ±5.9	159.1 ±4.5	166.7 ±6.3	156.8 ±3.9	165.7 ±8.2	152.4 ±4.4	163.5 ±5.7	154.6 ±4.2	166.6 ±6.8	154.7 ±4.1
3. FVC	3.7 ±0.6	2.9 ±0.4	3.7 ±0.4	2.6 ±0.2	3.7 ±0.3	2.8 ±0.5	2.7 ±0.3	2.0 ±0.2	3.6 ±0.5	2.6 ±0.5
4. %FVC	94.4 ±10.6	98.3 ±9.0	95.5 ±8.8	88.8 ±7.7	91.9 ±5.6	99.1 ±9.5	82.2 ±9.6	81.9 ±9.7	87.2 ±9.9	96.1 ±9.9
5. FEV_1	3.2 ±0.4	2.6 ±0.4	3.2 ±0.2	2.4 ±0.2	3.2 ±0.3	2.4 ±0.3	1.8 ±0.2	1.3 ±0.2	3.0 ±0.5	2.2 ±0.5
6. $\%FEV_1/FVC$	87.3 ±4.3	90.1 ±4.4	86.8 ±5.1	93.6 ±4.6	88.9 ±4.0	87.4 ±3.5	66.8 ±4.5	66.5 ±5.2	80.0 ±6.5	81.1 ±9.6

COPD = Chronic obstructive pulmonary disease.
FVC = Force vital capacity.
FEV_1 = Force expiratory volume at 1 second.

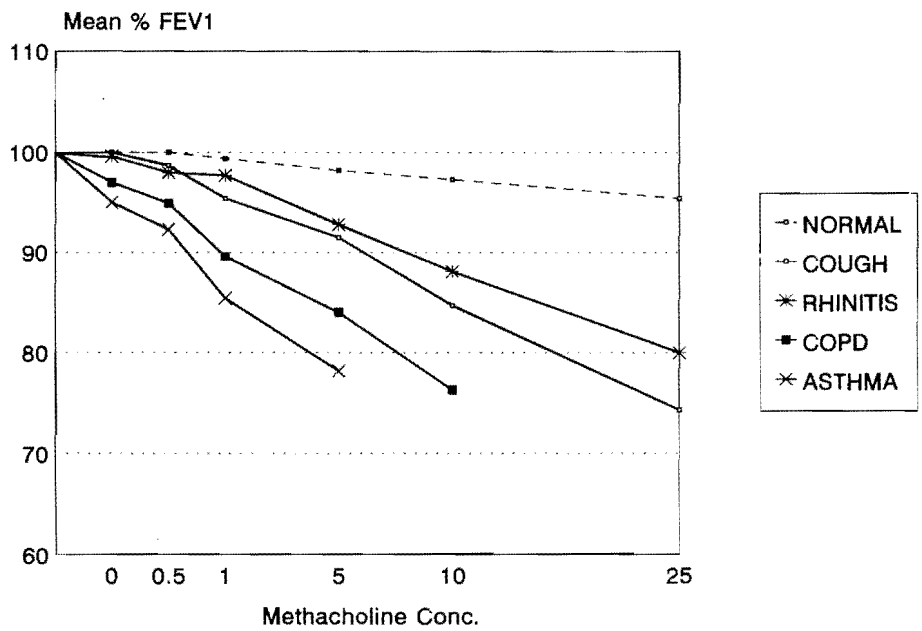

Fig. 1 Dose-response curve to methacholine inhalation.

meters, however, the difference was not statistically significant. Compared to other groups, the patients with COPD (group IV) were significantly older ($p < 0.05$) and had significantly lower values of spirometric parameters ($p < 0.05$).

There was no positive BHR in 20 non-smoker normal subjects. Everyone in the normal group could inhale all of each concentration of the methacholine without any side effects. The maximum decrease in FEV_1 in this group was 8% from the baseline FEV_1. In the group of patients with chronic cough, twelve (4 males and 8 females) showed positive tests (60%). Two normal subjects in group I developed upper respiratory tract infections, followed by chronic cough later and they were also included in group II 4–5 months after the first MIC test. One subject was found to have a positive BHR at 10 mg/ml of methacholine but another showed a negative result. No subject in group II responded to 0.5 and 1.0 mg/ml of methacholine. One subject (5%) showed a response at 5 mg/ml. Sixty percent of the chronic cough subjects were found to give a positive BHR at 25 mg/ml.

Table 2. Accumulative percentage of subjects responding to methacholine.

Methacholine concentration (mg/ml)	Normal subjects	Chronic cough	Allergic rhinitis	COPD	Asthma
0	NR	NR	NR	NR	NR
0.5	NR	NR	NR	NR	15%
1.0	NR	NR	NR	5%	35%
5.0	NR	5%	NR	40%	80%
10.0	NR	30%	10%	60%	85%
25.0	NR	60%	60%	95%	100%

Response = A decline of FEV_1 more than 20% from the baseline.
NR = No response.

In the group with allergic rhinitis, twelve (6 males and 6 females) showed positive BHR (60%). None of this group showed a positive test at 0.5, 1.0, and 5 mg/ml. Two patients (10%) responded to 10 mg/ml of methacholine. The others showed positive results at 25 mg/ml. Nineteen subjects of the COPD group (9 males and 10 females) showed positive BHR (95%). Twelve patients (60%) responded to 10 mg/ml of methacholine. One subject who was clinically stable with a lung function test of 70% FEV_1, showed decreasing FEV_1 of only 18% at 25 mg/ml of methacholine and this was considered to be a negative result. All subjects (100%) with asthma showed positive results. Sixteen patients (80%) of this group were also classified as having pronounced BHR. Only 3 asthmatic subjects could inhale 25 mg/ml of methacholine and the maximum decline of FEV_1 was 35% from the baseline.

From Fig. 1, the average values of the concentrations of methacholine causing a decline of FEV_1 greater than 20% (PC_{20}) were 15 mg/ml, 25 mg/ml, 7 mg/ml, and 4 mg/ml for groups II, III, IV and V, respectively. There were statistically significant differences in the PC_{20} between these various groups and normal subjects ($p < 0.05$).

Of the 100 subjects undergoing methacholine challenge test, 25 (25%) reported about adverse reaction symptoms due to inhalation of the methacholine. The symptomatic reaction consisted of cough (100%), shortness of breath (84%), wheezing (40%), dizziness (16%), headache (4%), and nausea (4%).

DISCUSSION

A knowledge of the behavior of aerosols is essential for the standardization of inhalation provocation tests. Tests with methacholine or histamine have become very useful in the clinical investigation of asthma, and their standardization is necessary so that the results can be accurately interpreted and compared in the same and different laboratories. Many factors are important, including aerosol generation and inhalation, method of measurement of response, and preparation and handling of test solution: pH, temperature, stability. The most important aspect of standardization is the method of aerosol generation and inhalation. The optimal method will be the one that is cheapest and simplest for the patients and the technician, and that will give reliable and reproducible results.

Numerous methods of aerosol generation and inhalation have been used. In the most widely used method, described by Chai *et al.*,[8] aerosol was generated intermittently by a de Vilbiss # 646 nebulizer connected to a Rosenthal-French dosimeter. The reservoir method has been recently found to have some exellent, reproducible results due to the stable deposition of methacholine in the bronchial tree. This technique has the advantage of simplicity for patients and technician, and of low cost.

The result of this study confirmed that MIC by a reservoir method was simple and reproducible. In the group of patients with chronic cough, 60% had positive result which was similar to the previous study.[9] There were many causes of chronic persisting cough. It may be due to chronic sinusitis with postnasal drip, chronic bronchitis and miscellaneous disorders. Cough may be the only presenting symptom of patients with asthma. However, most patients selected into this study did not have asthmatic attacks previously, and they had been diagnosed as having viral upper respiratory tract infections before developing chronic cough. This finding confirmed the data reported by Empey *et al.*[10] concerning the mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. Irwin *et al*[11] also found that MIC was the single most useful laboratory test in diagnosing adult patients with chronic persistent cough. They concluded that history, physical examination and MIC diagnosed disease in 86% of all patients. Cockcroft *et al.*[12] reported that there was increased bronchial reactivity in 47% of subjects with the isolated symptom of chronic recurrent cough which may be the

only early symptom of asthma and bronchodilator therapy may relieve this symptom.

Sixty percent of the subjects with allergic rhinitis also showed an increased bronchial reactivity. This result was similar to the data reported by Townley *et al.*[13] which demonstrated that 63% of hay fever patients had positive responses. The increased BHR in these patients was not as severe as in asthma. However, some patients with allergic rhinitis may also have had symptoms of asthma which were more likely to reflect hyperreactive airways than in those with rhinitis alone.[14] We did not include subjects who had both symptoms of rhinitis and asthma in the study. Felarca and Itkin[15] demonstrated slightly greater changes in the FEV_1 in subjects with hay fever than in normal subjects at high dose MIC. Our study also indicated that the response to methacholine of patients with allergic rhinitis occurred at high doses (10% and 60% of patients at 10 mg/ml and 25 mg/ml, respectively).

We found that 95% of patients with chronic obstructive pulmonary disease showed positive response to MIC. Sixty percent of these patients had positive response at 10 mg/ml methacholine. Ramsdell *et al.*[16] reported that all of 22 patients with chronic obstructive bronchitis were sensitive to inhaled methacholine, reacting at a dose of 4.29 ± 5.40 cumulative units. There were no normal responses. Those data suggested that airway reactivity may contribute to acute, transient exacerbations experienced by these patients, even in the absence of acute improvement in pulmonary function after the administration of sympathomimetics and may warrant chronic prophylactic bronchodilator therapy. However, a recent study demonstrated that chronic usage of sympathomimetics may also increase bronchial hyperresponsiveness.[17]

Extreme sensitivity of the airways to physical, chemical, and pharmacologic stimuli is a characteristic feature of asthma. As shown in Table 2, 100% of all patients had positive results for MIC, even those with no symptoms and normal lung function. Townley *et al.*[18] reported that over 90% of asthmatics had high or medium positive responses to methacholine. Only a few cases were negative and they were former asthmatics who had been completely free of symptoms for several years. The BHR in patients with asthma is persistent but it is not fixed because most asthmatic patients who ceased to have attacks remain methacholine challenge positive for many years after their last attack, though the degree of their sensitivity was only 1/10 that of current asthmatics. Possible pathogenesis of BHR is by airway inflammation and epithelial damage by various mechanisms involving cellular infiltration and release of severeal mediators.[19] Many drugs have been used to treat airway inflammation and to reduce the BHR, but corticosteroids are the most effective.[20]

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