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Methacholine inhalation challenge (MIC) using a reservoir method was performed at the Respiratory Unit, Chulalongkorn Hospital. Forty subjects, comprising 20 non-smoking healthy subjects and 20 with mild bronchial asthma, were scheduled to perform the test. Ten litres of methacholine aerosol were produced from 6 ml of methacholine solution in an atomizing nebulizer (Provocationtest I, Pari-Starnberg, Germany); the 10 litres of the aerosol was kept in a reservoir bag, from which the subjects inhaled the drug. Each subject inhaled the mist via slow vital capacity manuver. Increasing concentrations (0,0.5,1, 5,10,and 25 mg/ml) of the methacholine solution were used. The best spirometric maneuvers were performed before and after each methacholine inhalation. The highest FEV1 levels obtained from acceptable maneuvers were used for analysis. The procedure was continued with increasing concentrations of methacholine up to the maximum concentration of 25 mg/ml, unless there was a decline of more than 20% from the baseline value in the FEV1 level following any inhalation. In such a case, the test would be terminated. At the end of the test, the subjects in whom there was a decline in FEV1 of more than 15% would be administered an inhaled bronchodilator. None of the healthy subjects had a positive response. All (100%) of the patients with asthma had bronchial hyperresponsiveness (BHR) with a mean PC20 of 4 mg/ml. No serious effects were detected during or after the methacholine tests. Patients with mild asthma had a moderate degree of BHR.

Key words : *Methacholine inhalation challenge, Bronchoprovocation, Bronchial asthma.*

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สมเกียรติ วงษ์ทิม, สมคิด หมอกมืด, ประดิษฐ์ เจริญลาภ. การทดสอบการหายใจด้วยเมทาโคลีนในผู้ป่วยหอบหืดและคนปกติโดยวิธีถุงเก็บละออง. จุฬาลงกรณ์เวชสาร 2537 ธันวาคม; 38(12): 733-741

ได้ทำการทดสอบความไวของหลอดลมโดยการหายใจด้วยเมทาโคลีนที่ทำเป็นละอองฝอยอยู่ในถุงเก็บที่หน่วยโรคระบบทางการหายใจ โรงพยาบาลจุฬาลงกรณ์ จำนวนผู้ได้รับเข้าทำการทดสอบ 40 คน ซึ่งเป็นคนปกติผู้ที่แข็งแรงจำนวน 20 คน และเป็นผู้ป่วยหอบหืดขั้นเล็กน้อยจำนวน 20 คน การทดสอบได้ใช้เครื่องมือโพรโวเคชั่นเทสต์ หนึ่ง ของบริษัท พารี่สตารนเบิร์กแห่งประเทศเยอรมัน น้ำยาเมทาโคลีน 6 มล. ใส่อยู่ในกระเปาะสำหรับที่ทำละอองฝอย เมื่อเปิดสวิทซ์เครื่องทำงาน อากาศจะถูกดูดเข้าไปทำให้น้ำยากลายเป็นละอองฝอยและลอยขึ้นเข้าไปเก็บอยู่ในถุงเป็นจำนวน 10 ลิตร ผู้ได้รับการทดสอบจะสูดเอาละอองฝอยนั้นเข้าไปในปอดโดยการหายใจเข้าออกลึก ๆ น้ำยาเมทาโคลีนที่ใช้นั้นมีความเข้มข้นตั้งแต่ 0, 0.5, 1, 5, 10 และ 25 มก/มล. ผู้ได้รับการทดสอบจะต้องเป่าสไปโรเมตรีย์อย่างถูกต้องก่อนและหลังการสูดละอองน้ำยา ถ้าผลการเป่าสไปโรเมตรีย์เอฟอีวีที่ 1 วินาทีหลังการสูดละอองไม่ลดลงมากกว่า 20% เทียบกับก่อนสูดละออง ผู้ได้รับการทดสอบจะได้รับการสูดน้ำยาจนกระทั่งความเข้มข้นสูงสุดคือ 25 มก/มล. จึงจะหยุดการทดสอบ และตัดสินว่าการทดสอบให้ผลลบ ถ้าผู้ใดเป่าสไปโรเมตรีย์ลดลงมากกว่า 20% ณ ที่จุดความเข้มข้นใด ๆ ก็ตามก็จะหยุดการทดสอบทันที และตัดสินว่าการทดสอบให้ผลบวก (หลอดลมมีความไวมาก) หลังจากเสร็จการทดสอบแล้วผู้ที่เป่าลมลดลงมากกว่า 15% จะได้รับยาขยายหลอดลมจนดีขึ้น ผลการทดสอบพบว่าคนปกติทั้งหมดไม่มีผู้ใดให้ผลบวก แต่ผู้ป่วยหอบหืดทั้งหมดให้ผลบวกต่อการทดสอบ โดยมีค่าเฉลี่ยความเข้มข้นของน้ำยาเมทาโคลีนที่ทำให้ค่าเอฟอีวีที่ 1 วินาที ลดลงต่ำมากกว่า 20% เท่ากับ 4 มก/มล. ไม่พบว่ามีผลข้างเคียงที่รุนแรงจากการทำการทดสอบ สรุปว่าผู้ป่วยโรคหอบหืดขั้นเล็กน้อยก็ยังมีสภาวะหลอดลมไวมากพอสมควร

Bronchoprovocation may be defined as the administration of a stimulus in a susceptible subject to produce bronchospasm, followed by measurement of the result by spirometry. Inhalation challenge is a method of testing for bronchial hyperreactivity (BHR) by inhalation by a patient sensitive to a specific antigen, or nonspecific pharmacologic agents such as histamine, methacholine and carbacholine.[1] The methacholine inhalation challenge (MIC) involves two methods: (a) determination of a dose-response curve to an increasing concentration of methacholine while keeping constant the number of breaths and volume of methacholine inhaled, and (b) keeping the concentration of methacholine constant while increasing the number of inhalations of methacholine.[2] The first method is currently more widely used. The standard method recommended by the American Academy of Allergy involves the use of a nebulizer and dosimeter. However, from radioactive Technitium studies, it is now known that most of the methacholine aerosol is in fact deposited above the glottis.[3] A reservoir method has been recently found to have some advantage over the standard method in terms of excellent results and reproducibility owing to the greater deposition of pharmacologic agents in the bronchial system.[4] There is no information about the bronchial challenge test by this method in Thailand. The present study was designed to evaluate BHR by this challenge method in patients with mild asthma.

Materials and Methods

Subjects

Forty subjects divided into two groups were scheduled for the MIC involving a reservoir method at the Respiratory Unit of Chulalongkorn Hospital. The first group comprised 20 non-smoker healthy subjects (group I), i.e. medical personnel and medical student volunteers. All were in good healty and had no familial history of atopy; such as, asthma and allergic rhinitis. The second group comprised 20 patients with mild bronchial asthma. They were selected from among patients attending the Chest and the Allergy Clinics. They were classified as having mild asthma, defined as presenting 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 in FEV1/FVC of less than 70% of the level predicted, they typically would show as improvement in FEV1 greater than 15% after inhalation of a bronchodilator (salbutamol) All subjects were asked to refrain at least 12-24 hours before testing from using corticosteroid, antihistamine, bronchodilator, caffeine and other drugs that could have interfered with the test.

Procedure

For each subject, Methacholine challenge testing was performed at 9 A.M. by the same technician who did not know any details about the diagnosis of the subjects. Stock 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 the solutions were stored for not more than three months at 4°C. Methacholine solution was aerosolized by using the Provocationtest I (Pari-Starnberg, Germany). The atomizer part of the equipment was filled with 6 ml of the methacholine solution. After the button was switched on and the volume of air was set at 10 litres, air was pumped into the atomizer part to produce methacholine aerosol. The methacholine aerosol floated up and filled a reservoir bag attached to the top of the atomizer. After the pumping with air stopped, the reservoir bag would be filled with 10 litres of methacholine aerosol. An estimated 0.4 ml of solution was used to produce 10 litres of aerosol. The aerosolized methacholine was kept in the reservoir bag for 1 minute to allow the precipitation of large-sized aerosol droplets. At this point, the aerosol was ready to be inhaled into the respiratory tract of the subjects.

Before methacholine inhalation was initiated, baseline spirometric tests were performed with the subjects standing and using the Autospiror Discom-21 (Chest Corporation, Tokyo, Japan). At least three satisfactory and two reproducible

spirometric maneuvers were required for the test, according to American Thoracic Society recommendations.[5] The largest FEV_1 value from an acceptable maneuver was used for the baseline FEV_1. Two minutes after performing the spirometric tests, each subject inhaled diluent aerosol from the reservoir bag via a slow inspiratory and expiratory vital capacity maneuver until the bag was empty. There was no need for the subject to hold his breath at the end of inspiration. It usually took about 1 minute (8-12 breaths) to complete the inhalation of the aerosol according to the vital capacity of the subject. Three minutes after the inhalation, spirometry was repeated to determine the best level. The largest FEV_1 from an acceptable maneuver was used as the post-diluent control value. If the decline of FEV_1 after diluent inhalation was more than 20% from the baseline, the test would be terminated; if the decline was 15-19%, only 5 litres of the first methacholine concentration would be administered; if the FEV_1 decline was less than 15% then all 10 litres of the first methacholine concentration would be inhaled. Three minutes after the first aerosolized methacholine inhalation, spirometry was repeated to determine the best level. If the decline of FEV_1 was less than 10% the procedure was continued with an increasing concentration of methacholine up to the maximum concentration of 25 mg/ml. 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% from the baseline FEV_1, the test would be terminated. If the decline of FEV_1 was 14-16%, only 5 litres of the next highest concentration of methacholine was given. If the decline of FEV_1 was 17-19%, 10 litres of the same concentration was given. If the FEV_1 still failed to decline more than 20% an additional 5 litres of the same concentration was inhaled and spirometry repeated. At the end of the test, the subjects who had a more than 15% decline of FEV_1 would be administered four puffs of salbutamol from a metered-dose inhaler via a spacer; spirometry was repeated 10 minutes later. Subjects were told about a possible late phase reaction occurring 6-8 hours after the test. They were discharged from the Clinic after their FEV_1 had returned to within 10% of their baseline value.

Data analysis

Subjects were classified as having BHR (positive to the test) it they showed a more than 20% decrease in FEV_1 (PC_{20}) from baseline after inhalation of any concentration of methacholine up to and including 25 mg/ml.[6] They were also defined 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 on a computer, using the Statistical Package for the Social Sciences Program (SPSS). Results were presented as the mean +/- standard deviation (SD). For comparision 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, Figure 1 and 2. The mean values plus standard deviation for age, height, FVC, %FVC, FEV_1, %FEV1/FVC in normal subjects and asthmatic patients are shown in Tables 1. There were no statistically significant differences in terms of age and height among the subjects in both groups. Asthmatic patients (group II) had slightly lower values for the spirometric parameters however, the differences were not statistically significant.

There was no positive BHR in the 20 non smoking normal subjects. Everyone in this group was able to inhale all of each concentration of methacholine up to 25 mg/ml without any effect. The maximum decreasing FEV_1 in this group was 5% from the baseline FEV_1.

All subjects (100%) with asthma showed positive results. Two patients (10%) responded to only 0.5 mg/ml of methacholine. Twelve patients (60%) showed positive response at 5 mg/ml and these were classified as having pronouced BHR. Four patients responded to repeated doses of 5 mg/

Table 1. Dermographic Characteristics and Lung Function Paramaters of the Asthmatic Patients Compared to Normal Subjects.

		Asthmatics	Normal	P value
1.	Sex (n=...)	20	20	NS
	- male	50%	50%	NS
	- female	50%	50%	NS
2.	Age (x±SD)	34.2 ± 8.9	33.0 ± 8.09	NS
3.	Height	163.3 ± 6.7	163.8 ± 8.7	NS
4.	FVC	3.32 ± 0.7	3.37 ± 0.67	NS
5.	FEV_1	2.78 ± 0.51	2.97 ± 0.51	NS
6.	$\%FEV_1/FVC$	84 ± 8.4	89 ± 4.6	NS
7.	PC_{20}	4.1 ± 3.05	>25	<0.05

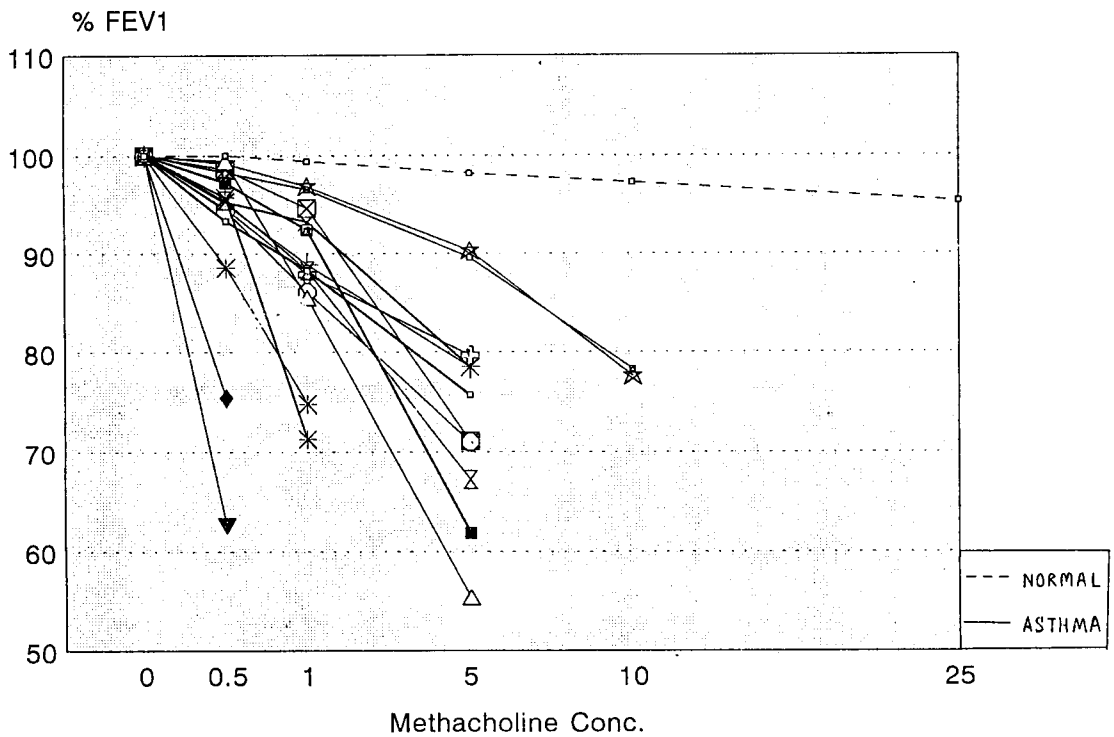

Figure 1. Percentage of FEV_1 decline from baseline observed with the methacholine challenge for normal subjects and asthmatic patients. Average concentration of methacholine causing 20% fall of FEV_1 (PC_{20}) for asthmatics was 4 mg/ml.

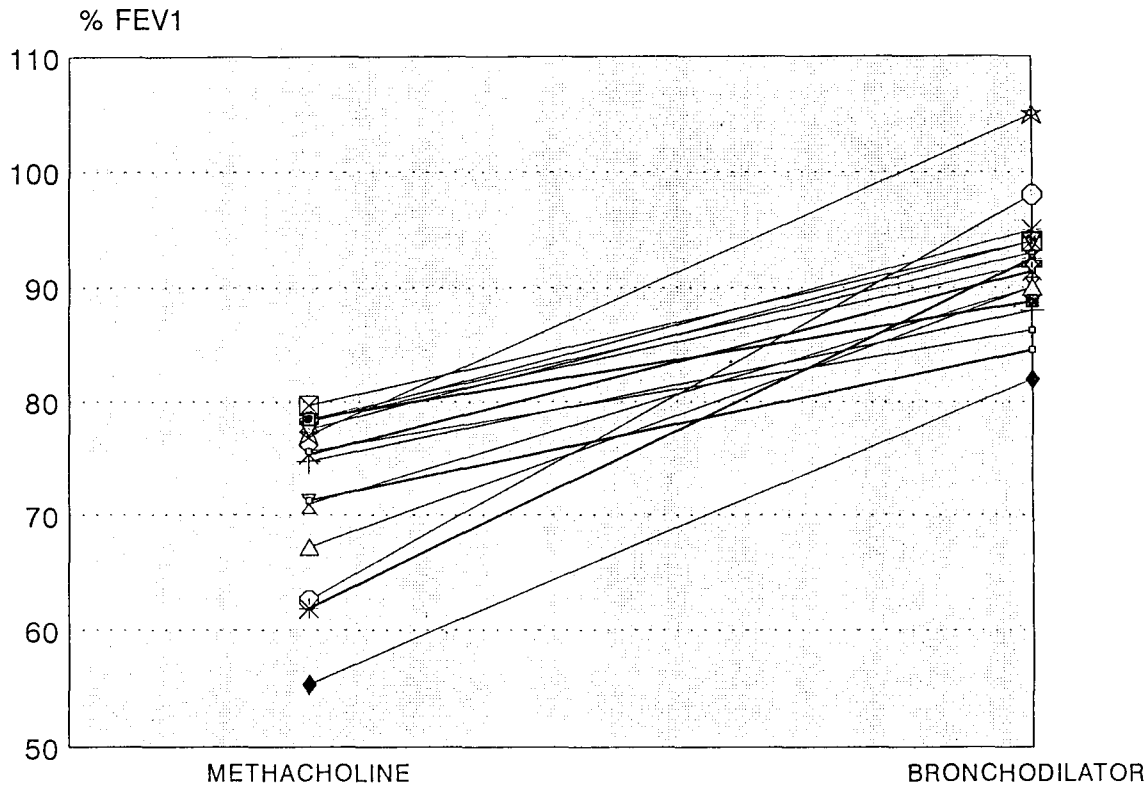

Figure 2. Maximal decrease in FEV_1 as a percentage of baseline FEV_1 after methacholine inhalation and subsequent improvement 10 minutes after inhaled salbutamol in asthnatic patients.

ml. Only two asthmatic subjects were able to inhale the methacholine aerosol up to 10 mg/ml. The maximum decline of FEV_1 was 44% from the baseline.

Percentages of the decline of FEV_1 from baseline in normal subjects and asthmatic patients are presented in Figure 1. The average value of the concentration of methacholine causing declines of FEV_1 greater than 20% (PC_{20}) in the asthmatic group was 4 mg/ml. Compared with normal subjects, the response to methacholine in asthmatic patients was statistically different ($p<0.05$).

In the asthmatic group, FEV1 fell to 73.1+11 percent of the baseline values. This bronchospasm was reversed and the FEV_1 returned to 92.3+9 percent in 10 minutes after inhalation of a bronchodilator; the difference was statistically significant (Figure 2).

Adverse reaction symptoms were reportd in 10 subjects with asthma, namely cough, shortness of breath and wheezing. No late phase reaction was reported.

Discussion

Inhalation provocation tests are now widely used in the investigation of various lung sieases, particularly asthma and other hpersensitivity lung diseases. The challenging agents can be divided into two main categories. First are agents which non specifically provoke bronchoconstriction in all potential subjects. These include cholinergic agnoists (acetylcholine, methacholine, carbachol), vasoactive amines (histamine) and arachidonic acid metabolites (leukotrienes). Vasoactive amines comprise the group used to determine specific (allergic) sensitization; they include common allergens such as the shedding of the house-dust mite.[7]

The measurement of non specific airway hyperreactivity is useful in the study of the pathogenesis of asthma. Asthma is currently described as a chronic lung disease with the following characteristics: (1) airway obstruction that is reversible (but perhaps not completely in some patients) either spontaneously or with treatment; (2) airway inflammation; and (3) increased airway respon-

siveness to a variety of stimuli.[8] The characteristic feature of asthma is bronchial hyperresponsiveness (BHR), a condition manifested by an exaggerated bronchoconstrictor response to many physical changes, and chemical and pharmacological agents.[9] Asthmatic patients develop clinical symptoms such as dyspnea and wheezing after exposure to allergens, environmental irritants, viral infections, cold air, or exercise. BHR also appears to be important in the pathogenesis of asthma, as it is ubiquitous in the disease.[10] Furthermore, the level of BHR usually correlates with the clinical severity of asthma and medication requirements.[11]

Numerous methods of methacholine inhalation challenge have been used. The standard method., decribed by Chai et al, is methacholine aerosol generated intermittently by a deVilbriss # 646 nebulizer connected to a Rosenthal-French dosimeter.[12] This method is very complex and it deposits most of the methacholine aerosol above the glottis. A new method of aerosol gradation with storage of the mist in a reservoir bag has been found to produce excellent results. Careful attention to standardization of this method has been recognized in order to achieve reproducibility and comparability of results. It has been found that the methacholine inhalation challenge by this reservoir method is simple and reproducible.

The clinical usefulness of bronchial inhalation testing with methacholine is apparent in four areas: (1) the diagnosis of asthma, (2) assessment of asthma severity, (3) diagnosis and follow-up of occupational asthma, and (4) monitoring and assessing the results of treatment.[13]

We found that methacholine inhalation challenge was useful in the diagnosis of asthma. Response to methacholine inhalation in our patients was positive in moderate to severe degrees, with the mean value of PC_{20} being 4 mg/ml. Cockcroft stated that a histamine PC_{20} greater than 8 mg/ml virtually ruled out current symptomatic asthma; a PC_{20} below 2 mg/ml was virtually diagnostic of current symptomatic asthma.[14] However, an arbitary "cutoff" point was difficult to define because some patients with allergic rhinitis, cystic fibrosis, and chronic obstructive pulmonary disease, as well as normal subjects, especially following airway injury caused by viral infection or exposure to oxidants, may also respond to the inhalation challenge, but to a lesser degree than patients with asthma. When history, physical findings, and spirometry were not adequate to confirm the diagnosis of asthma. These situations included cough-varients asthma and occupational asthma.

Townley reported that over 90% of asthmatics had high or medium positive responsiveness to methacholine. Only a few cases of asthma had negative response to the test, but these were former asthmatics who had been completely free of symptoms for several years.[15] Airway hyperresponsiveness in patients with asthma was persistent but not fixed. Although asthmatic patients who had higher levels of BHR. tend to have more severe asthma, there were individual variations, and some patients with relatively mild asthma demonstrated high levels of BHR. Most asthmatics who ceased to have attacks remained methacholine-challenge-positive for many years after their last attack, though the degree of their sensitivity was only 1/10 that of current asthmatics.

In the assessment of the severity of asthma and the guidelines for therapy, bronchial responsiveness to methacholine generally correlated quite closely with various aspects of assessment of the severity of asthma. It had been suggested that a patient with mild asthma would have a methacholine PC_{20} of more than 20 mg/ml; one with moderate asthma, a PC_{20} between 2 and 20 mg/ml; and one with severe asthma, a PC_{20} of less than 2 mg/ml.[16] While the degree of methacholine sensitivity generally correlated with the severity of symptoms and medication requirement, there were exceptions as individual variations. Our patients with mild asthma had a methacholine PC_{20} of only 4 mg/ml, which is less than the PC_{20} value reported in the literature. This may be due to asthmatic Thai subjects (1) having tolerance to symptoms, or (2) having more sensitivity to methacholine. However, this situation may also be due to the selection of cases for study as only symptomatic criteria were used. Pulmonary function may in fact be included in case selection

because patients with mild asthma should have a PEFR more than 80% predicted, and variability should be less than 20% during each day for a week. However, our asthmatic patients still had mild symptoms after the tests and they also required the same medication.

To assess the outcome of treatment, serial methacholine inhalation challenge could also be used to follow the patients and to monitor the results of treatment. Avoidance of allergen or occupational sensitizing agents and long-term use of sodium cromoglycate or corticosteriod may have a beneficial effect on bronchial responsiveness.[17,18] Allergen hyposensitization may also improve non-specific bronchial hyperresponsiveness, but the results are less convincing at this moment. It has been suggested that new treatments for asthma should also be monitored with regard to their effects, both short-term and long-term, on non-specific bronchial hyperresponsiveness.[7]

The regimens of treatment in patients with mild asthma include inhaled bronchodilator when the patients have symptoms. However, inhaled B2 agonist or anticholinergic drugs have no effect on the improvement of BHR. There have been some reports showing an improvement in BHR in patients with mild to moderate asthma after they received inhaled budesonide,[19,20] but others have not found that improvement.[21,22]

In conclusion, patients with mild asthma still had a moderate degree of BHR and they should be administered with anti-inflammatory drugs, including inhaled corticosteroid, to decrease BHR. However, further studies should be performed to improve carrent knowledge in this area.

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References

1. Rosenthal RR. Inhalation chaloenge: procedure, indication, and technique. The emerging role of bronchoprovocation. J Allergy Clin Immunol 1979 Dec; 64(6pt 2):564-8
2. Townley RG, Dennis M, Itkin IH. Comparative action of acetyl-beta-methyl choline, histamine and pollen antigens in subjects with hay fever and patients with asthma. J Allergy 1965 Mar-Apr; 36(2):121-6
3. Reed CE, Bhansali PV, Irvin C, Polcyn RE. Site of deposition of aerosols delivery by two methods using the dosimeter. J Allergy Clin Immunol 1979 Mar; 63(3):164-9
4. Klein G, Kohler D. Standardization of inhalation challenges for measuring the unspecific bronchial inhalation challenge by a reseveroir method. Pneumologie 1991; 45:1-4
5. American Thoracic Society. Medical Section of the American Lung Association. Standardization of spirometry-1987 update. Am Rev Respir Dis 1987 Nov; 136(5):1285-98
6. Tashkin DP, Altose MD, Bleecker ER, Connett JE, Kanner RE, Lee WW, Wise R. The lung health study: airway reponsive-ness to inhaled methacholine in smokers with mild to moderate airflow limitation. Am Rev Respir Dis 1992 Feb; 145(2):301-10
7. Cockcroft DW. Bronchial inhalation test I. Measurement of nonallergic bronchial reponsiveness. Ann Allergy 1985 Oct; 55(4):527-33
8. American Thoracic Society. Chronic bronchitis, asthma, and pulmonary emphysema. Am Rev Respir Dis 1987; 136:225
9. Bleecker ER. Airways reactivity and asthma: significance and treatment. J Allergy Clin Immunol 1985 Jan; 75(1):21-4
10. McFadden ER JR, Gilbert IA. Asthma. N Engl J Med 1992 Dec 31; 327(27):1928-7
11. Cockcroft DW. Airway hyperresponsiveness: therapeutic implications. Ann Allergy 1987 Dec 59(6):405-14

12. Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL, Spector SL, Townley RG. Standarization of bronchial inhalation challenge procedures. J Allergy Clin Immunol 1975 Oct; 56(4):323-7
13. Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial hyperreactivity. Am Rev Respir Dis 1980 Feb; 121(2):359-413
14. Cockcroft DW, Berschield BA, Murdock KY, Gore BP. Sensitivty and specificity of histamine PC20 measurements in a random population. J Allergy Clin Immunol 1985 Jan; 75(1):142 Suppl
15. Townley RG, Ryo UY, Kolothin BM, Kang B. Bronchial sensitvity to methacholine in current and former asthmatic and allergic rhinitis patients and control subjects. J Allergy Clin Immunol 1975 Dec; 56(6):429-35
16. National Asthma Education Program. Guidelines for the diagnosis and management of asthma. U.S. Department of Health and Human Services; National Institute of Health, August 1991
17. Platts-Mills TAE, Mitchell E, Nock P, Tovey ER, Moszoro H, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982 Sep 25; 2(8300):675-7
18. Clissold SP, Heel RC. Budesonide: a preliminary review of its pharmacodynamic properties and therapeutic efficacy in asthma and rhinitis. Drugs 1984; 28(6):485-518
19. Juiper EF, Kline PA, Vanzieleghem MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. Am Rev Respir Dis 1990 Oct; 142(4):832-6
20. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Time course of change in bronchial reactivity with inhaled corticosteroid in asthma. Am Rev Respir Dis 1991 Jun; 143(6):1317-21
21. Kraan J, Koeter GH, Van der Mark Tw, Boorsma MM, Kukler J, Sluiter HJ, de Vries K. Dosage and time effect of inhaled budesonide on bronchial hyperreactivity. Am Rev Respir Dis 1998 Jan; 137(1):44-6
22. Kivity S, Fireman E, Greif J, Schwarz Y, Topilsky M. Effect of budesonide on bronchial hyperresponsiveness and pulmonary function in patients with mild to moderate asthma. Ann Allergy 1994 Apr; 72(4):333-6