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STANDARDIZATION OF METHACHOLINE INHALATION CHALLENGE*

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Methacholine inhalation challenge has been shown to be an extremely useful diagnostic test. The purpose of this study was to document the reproducibility of methacholine inhalation challenge used in our clinic. In addition, we also examined the output of the delivery system, the stability of four-month-old methacholine solution, and the cumulative effect of methacholine.

To document reproducibility, two identical challenges were performed in each of 19 asthmatic children. The influence of the previous doses of methacholine on bronchial response was examined by performing a third challenge with a single dose of methacholine in ten of these children. The remaining nine children were also tested for the third time with four-month-old methacholine solution to examine the stability of that solution. The output of the delivery system was assessed by measuring the change in weight of the nebulizer. Responses to methacholine were highly reproducible within one doubling dose interval. There was a small but significant cumulative effect of methacholine. Comparable results were obtained with newly prepared methacholine solution and four-month-old solution. The variability of the output of the same nebuliser was less than that of different nebulisers of the same model.

The major clinical implication of our results is that our methacholine inhalation challenge procedure is standardized. This encourages more widespread use of this important diagnostic test for demonstration of airway hyper-responsiveness. *Key words: methacholine inhalation challenge, children, airway responsiveness, bronchial asthma.*

The narrowing of airways in response to various stimuli, such as methacholine, histamine, adenosine, exercise and cold, dry air, is called airway responsiveness (AR). In various respiratory problems, an individual's response to these agents may be increased; these individuals are said to have airway hyper-responsiveness (AHR)[1].

A quantitative assessment of the presence and degree of AHR can be made by measuring the response to standardized stimuli. Most tests are performed by

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administration of an agonist in the form of an aerosol followed by measurement of lung function. The most commonly used nonspecific agents are methacholine (Mch) and histamine[2].

Patients with asthma have AHR. Measurement of AR is widely used to confirm the presence of asthma, quantify the severity of the disease and assess the response to anti-inflammatory therapy[3].

This study was designed to define the short-term reproducibility of the methacholine inhalation challenge test used in our clinic. We also examined the cumulative effect of Mch and the stability of methacholine solution after four months of storage and described nebuliser output variability.

Material and Methods

Study Population

Nineteen asthmatic children currently being followed at the Pediatric Allergy Unit of Hacettepe University İhsan Doğramacı Children's Hospital participated in the study. All had episodic dyspnea, wheezing and reversible airway obstruction following bronchodilator therapy. At the time of study their symptoms were well controlled, and their forced expiratory volumes in one second (FEV1) were greater than 70 percent of their predicted values. FEV values did not vary by more than five percent on each study day. There was no history of asthma exacerbation or respiratory tract infection in the four weeks preceding the first study. Aerosol bronchodilators were withheld for 12 hours and oral bronchodilators for 24 hours before each study day.

Test Procedure

Our methacholine inhalation challenges were carried out by a method similar to that described by Cockcroft et al[4]. In each test an aerosol of saline was inhaled first, followed five-minute intervals by inhalation of methacholine in two-fold increasing concentrations, from 0.03 to 8 mg/ml. Salbutamol aerosol was administered to obtain recovery following completion of each challenge.

The aerosol was produced by a DeVilbiss 646 (Somerset, PA, USA) nebuliser which was operated by DeVilbiss air compressor (Pulmo-Aid, Somerset, PA, USA). On each occasion 4 ml of diluent or normal saline was placed into the nebuliser. The patient, wearing a nose clip, inhaled the aerosol through a mouthpiece for two minutes during tidal breathing. The same nebuliser was used for each challenge. The response, measured as the change in FEV_1 with the child in a standing position, was assessed using a dry rolling seal spirometer (SensorMedics 2130 Spirometer, California, USA). Initially, the forced expiratory maneuvers were repeated until three reproducible FEV_1 values were obtained.

After each inhalation, FEV_1 was measured at 0.5 and 1.5 minutes, and if necessary at three minutes to obtain the lowest post-inhalation FEV_1. The FEV_1 was measured only once on these occasions unless the measurement was technically unsatisfactory. Inhalations were continued until the post saline FEV_1 had fallen by 20 percent or more, or until a concentration of 8 mg/ml had been administered.

The responses were expressed in terms of the provocative concentration of methacholine required to produce a 20 percent fall in FEV_1 (PC20). This was obtained from the log dose response curve by linear interpolation of the last two points.

Solutions of methacholine were made from methacholine chloride powder and normal saline, as reported previously[5]. The prepared solution of 32 mg/ml methacholine was filtered using a 0.22 µm filter. Four ml of the solution was then placed in a sterile, single-use, black-covered vial and stored at 4 °C until the day of the challenge. To assess the stability of four-month-old Mch solution, ten of these vials were used in further challenges after four months. Thirty minutes before each challenge, the solution was diluted with normal saline and then placed in a sterile, single-use vial marked with a different color.

Study Design

The short-term reproducibility of the test was investigated in a single-blind manner by two identical challenges in each of the 19 patients. The challenges were performed at the same time on two of three days.

Nine patients, each with a PC20 greater than 0.25 mg/ml, underwent a third test to determine the cumulative dose effects of Mch. Initial saline inhalation was followed by inhalation of the final concentration of that individual's first Mch challenge. The PC20 obtained from a single-dose challenge of Mch [PC20(s)] was calculated by the following formula:

$$PC20(s) = \frac{\text{Concentration of Mch X 20}}{\%\ \text{fall in } FEV_1}$$

In the remaining ten patients, the original challenge was repeated for a third time (PC20(4m)) with four-month-old Mch solution to assess its stability following storage. PC20(4m) was then compared with the first challenge (PC20(1)).

The output of the three nebulisers (A, B and C) was determined as fallows: 4 ml of normal saline was placed in each nebuliser which was then operated with a DeVilbiss compressor (Pulmo-Aid, Somerset, PA, USA) for two minutes. Output was determined by measuring the resultant change in weight of the nebulises. The mean of trials was calculated to determine the individual variability of each nebuliser and the variability between nebulisers of the same model.

Statistical Analysis

The results of the methacholine inhalation challenges were analyzed after logarithmic transformation of each PC20 value. Spearmann rank correlation, Wilcoxon test and coefficient of variation were used for statistical analysis. A significance level of $p = 0.05$ was used.

Results

The age of the children ranged from seven to 16 years with a mean age of 11.37 ± 0.7 years. Each child underwent three challenges on three out of seven days. The first two were identical challenges to estimate the reproducibility of the test. A third challenge was performed in 10 children with four-month-old Mch solution stored at 4 °C, and in the remaining nine children with signle-dose Mch. The results of the challenges are shown in Table I.

Table I: Results of Methacholine Inhalation Challenges

Case No.	Age	Sex	PC20(1)	Pc20(2)	Pc20(s)	Pc20(4M)
1	16	F	0.11	0.18	0.22	
2	13	M	0.35	0.22	0.5	
3	13	M	3.1	3.07	5	
4	10	F	0.33	0.45	0.55	
5	10	M	3.4	2.3	3.63	
6	14	F	3.24	4.01	5.7	
7	11	F	1.2	1.75	2.9	
8	12	M	1.9	1.6	2	
9	14	F	0.59	0.7	1	
10	12	F	0.1	0.07		0.11
11	7	M	0.1	0.08		0.13
12	8	F	0.17	0.13		0.3
13	9	M	0.14	0.21		0.18
14	8	M	0.61	0.46		0.81
15	14	M	5.66	6.4		4.4
16	8	M	0.26	0.3		0.13
17	6	F	0.66	0.91		1.1
18	16	F	0.17	0.16		0.21
19	15	F	0.91	0.75		1.23

There was a high degree of reproducibility in response to methacholine [PC20(1) and PC20(2)] with a coefficient of determination (r^2) of 0.98. The measurements were within the tow-log-dose response, as shown in Figure 1. Inhalation of at least four doubling concentrations of methacholine at intervals of five minutes

produced a cumulative effect in every subject in that all the PC20 values obtained by the single-dose challenge [PC20(s)] were lower than the first individual PC20 values [PC20(1)], as shown in Figure 2. For the group the difference was significant (Wilcoxon test, $p < 0.05$).

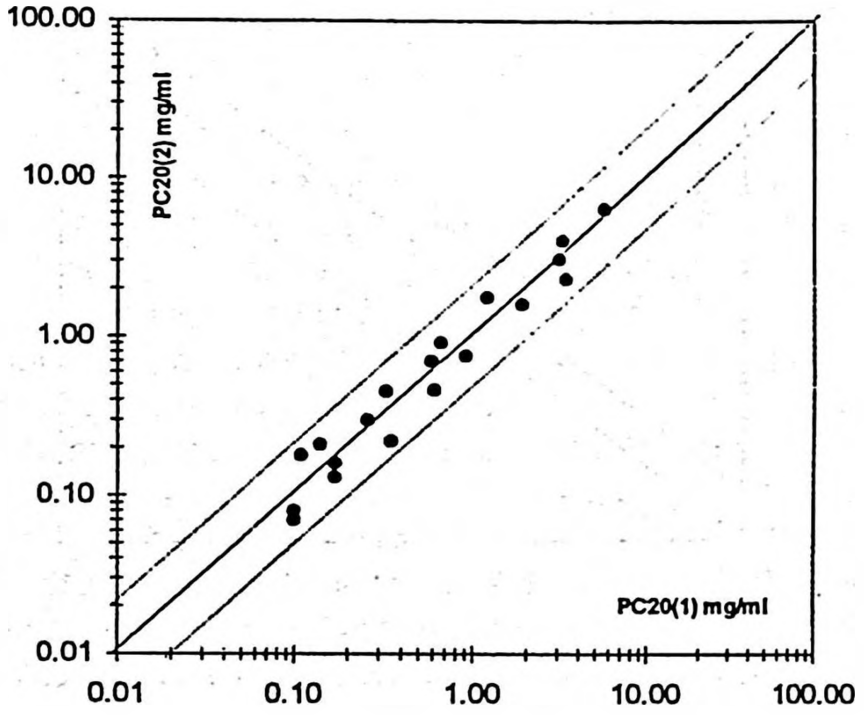

Fig. 1: Relation between the PC20 of the first and second tests. The solid line is the line of identity, and dotted lines indicate one doubling dose interval from the line of identity.

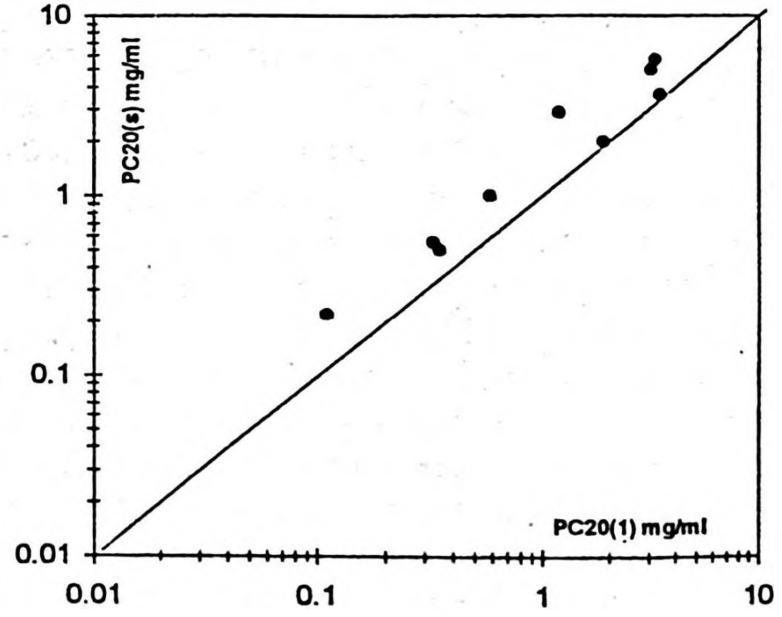

Fig. 2: Relation between the PC20 of the first test and the single-dose test. The solid line is the line of identity.

There was a significant coefficient of determination ($r^2 = 0.99$) between PC20 values obtained with fresh prepared [PC20(1)] and four-month-old [PC20(4m)] methacholine challenges. The two challenges are displayed in Figure 3. For the group the difference was not significant (Wilcoxon test, $p > 0.05$).

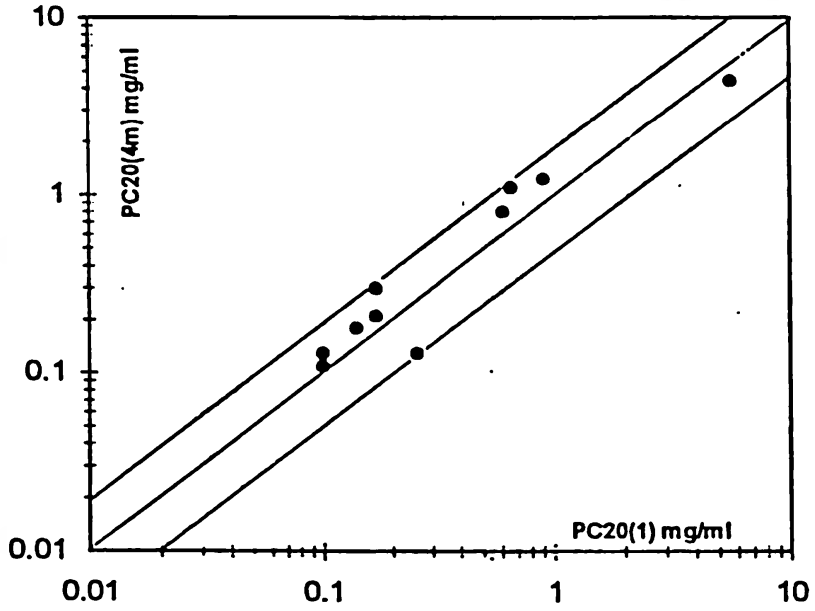

Fig. 3: Relation between the PC20 of the first and third tests with four-month-old Mch. The solid line is the line of identity, and the dotted lines indicate one doubling dose interval.

The aerosol outputs of DeVilbis 646 nebulisers, which were operated with a consistent compressor, are shown in Table II. The variabilities (coefficient of

Table II: Nebuliser Output

Nebuliser	Nebuliser Output (ml/min)	Mean ± SD (ml/min)
A	0.240 0.234 0.242 0.228 0.242	0.234 ± 0.009
B*	0.264 0.268 0.260 0.260 0.258	0.262 ± 0.008
C	0.272 0.266 0.270 0.270 0.272	0.270 ± 0.008

*Nebuliser used in all challenge tests.

variation) of different nebulisers from the same model were 3.75, 3.1 and 3.13 percent for nebulisers A, B, and C, respectively. The variation increased to 6.9 percent when all three nebulisers were taken into account.

The majority of children had no difficulty in complying with the test. There was only one child with technically unsatisfactory data because of a communication problem (data not included).

Discussion

For valid interpretation of test results (in this case the concentration of Mch producing a 20 percent decrease in FEV_1), it is essential to know the degree of variability within the involved test. To this end, we carried out a study on 19 asthmatic children by performing two identical Mch challenges to document the reproducibility of this test. Highly reproducible results at a one-doubling-dose interval were obtained, suggesting our procedure is technically standardized. The results of previous reports studying the variability of this method did not differ from ours[4, 6]. Aside from technical variables, individual variables also affect the results. Since subjects with mild to moderately severe asthma were included in our study, the variability might also be affected by individual variation, which suggests that our results may be applicable to clinical practice.

The dosimeter technique is the other method for measurement of AR. In this method, which was established by Chai et al[7], a dosimeter is used to deliver a consistent amount of agonist during the first 0.6 seconds of each of five inspiratory capacity breaths. It has been concluded that both tidal breathing and the dosimeter method are suitable for measurement of AR in children and adults[6, 8]. We prefer the tidal breathing method, since less cooperation and equipment (dosimeter) are required. The main disadvantages of our method are the following: first, the test takes more time than the dosimeter method, and second, it is not possible to be sure of the actual dose delivered to the patient. The results of the nebuliser output study demonstrate that the variation of output in each individual nebuliser was approximately four percent, increasing to seven percent when different nebulisers of the same model are used. As reported previously[9], nebuliser output must be known and kept constant to avoid becoming a variable in the inhalation test. Using different nebulisers of the same model may increase the variability of the test, so the results may not be interpreted as accurately. The output of nebulisers was also shown to be determined by the operating flow rates[9]. To eliminate this technical factor, we prefer to use an air compressor rather than a central air source. In the latter situation, the respiratory technician would adjust the air flow, and differences in this adjustment would increase the variation of the challenge tests.

Two other conclusions that merit comment emerged from our results. First, Mch has a cumulative effect. Therefore, shorter test protocols may increase the variability of the challenge when compared to a full protocol. Second, Mch solution of 32 mg/ml is stable after four months when refrigerated at 4 °C, which indicates that Mch solution needs to be made no more frequently than every four months. This finding is also in agreement with Pratter et al[10].

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