ORIGINAL ARTICLES

Bronchial Hyperresponsiveness to Methacholine Assessed by Means of Tracheal Auscultation of Healthy Children Aged Under 4 Years

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OBJECTIVE: To assess the safety of the tracheal auscultation method for measuring bronchial hyperresponsiveness in healthy unsedated children aged less than 4 years and to establish a range of normal bronchial hyperresponsiveness values.

POPULATION AND METHODS: The study population consisted of healthy children aged between 6 months and 4 years. A methacholine bronchial provocation test was administered to unsedated children, using the tidal volume breathing technique and applying an abbreviated protocol. The test was considered positive when wheezing was heard in the trachea, arterial oxygen saturation (SaO_2) fell by 5% or more, or respiratory rate increased by 50% or more.

RESULTS: A total of 16 children were studied. Ages ranged from 8 to 47 months, with a mean (SD) of 23.5 (12.2) months. There was no response to the methacholine in 11 children. In the other 5 children, there was a positive response at a concentration of 8 mg/mL. Response to the test was considered positive on the basis of tracheal wheezing in 3 cases, tracheal wheezing and a fall in SaO_2 in 1 case, and a fall of more than 5% in SaO_2 in 1 case. SaO_2 never fell below 93%.

CONCLUSIONS: As a means for assessing bronchial hyperresponsiveness, the tracheal auscultation method is appropriate, is simple to apply, and can be safely administered to unsedated children aged less than 4 years. The lowest concentration at which a response to methacholine occurs in healthy children of this age group is 8 mg/mL.

Key words: *Bronchial hyperresponsiveness. Tracheal auscultation. Methacholine. Children.*

Estudio de la hiperrespuesta bronquial a la metacolina mediante la auscultación traqueal en niños sanos menores de 4 años

OBJETIVO: Valorar la seguridad del método de la auscultación traqueal para medir la hiperrespuesta bronquial en niños sanos menores de 4 años de edad, sin sedación, y establecer valores de normalidad.

POBLACIÓN Y MÉTODOS: Se incluyó en el estudio a niños sanos de entre 6 meses y 4 años de edad. Se les realizó una prueba de provocación bronquial con metacolina mediante la técnica de la respiración a volumen corriente, en niños no sedados, utilizando un protocolo acortado. Se consideró positiva la prueba cuando se auscultaron sibilantes en la tráquea, la saturación arterial de oxígeno (SaO_2) disminuyó un 5% o más, o bien aumentó la frecuencia respiratoria un 50% o más.

RESULTADOS: Se estudió a 16 niños de 8 a 47 meses de edad (mediana: 23,5 meses; desviación estándar: 12,2 meses). En 11 niños no hubo respuesta a la metacolina, y en 5 la respuesta fue positiva a la concentración de 8 mg/ml. La prueba se consideró positiva en 3 casos por la auscultación de sibilancias traqueales, en uno por la auscultación de sibilancias y descenso de la SaO_2, y en otro por un descenso de la SaO_2 del 5% o superior. En ningún caso la SaO_2 disminuyó por debajo del 93%.

CONCLUSIONES: El método de la auscultación traqueal es sencillo, adecuado y seguro para valorar la presencia de hiperrespuesta bronquial en niños menores de 4 años de edad, sin necesidad de sedarlos. La concentración mínima a la que los niños sanos de esta edad responden a la metacolina es 8 mg/ml.

Palabras clave: *Hiperrespuesta bronquial. Auscultación traqueal. Metacolina. Niños.*

Introduction

Childhood asthma is a heterogeneous disease, with three types of syndrome involving wheezy bronchitis[1]: *a)* early, transient wheezy bronchitis associated with respiratory infections, and occurring in the first 3 to 5 years of life; *b)* nonatopic wheezy bronchitis related to viral infections, and possibly persisting until the age of 11 to 13 years (nonatopic asthma); and *c)* wheezy bronchitis associated

with allergic sensitization, and corresponding to the classical asthma phenotype (atopic asthma).

The incidence of recurring wheezy bronchitis in children under 4 years old is very high—in some studies as high as 34%.[2] Around 60% of these children belong in the transient wheezy bronchitis group and 20% in the nonatopic asthma group; the remaining 20% will eventually be diagnosed as having atopic asthma.[3]

Diagnosis of asthma in children in this age group is currently based on an assessment of clinical symptoms and of family and personal histories of allergies.[4,5] Given the importance of early detection of asthma, research is being conducted in a number of areas—epidemiology, respiratory function, and inflammation markers—with a view to providing early treatment to children at high risk of developing lifelong asthma.

Bronchial hyperresponsiveness, which is a characteristic feature of asthma in both adults and older children, is closely linked to the severity of wheezing bouts in children aged 8 to 15 years[6]; however, the same association has not been conclusively demonstrated for children aged 0 to 6 years.[7]

A diagnosis of asthma based on lung function testing relies on evidence of bronchial hyperresponsiveness; this is difficult to measure in small children, however, as they cannot undergo the lung function or bronchial challenge tests that are used with older children and adults.

Bronchial challenge with methacholine is used to assess bronchial hyperresponsiveness in older children and adults. In this test, the reduction in forced expiratory volume in 1 second (FEV_1) is evaluated as the response variable. This test has not, however, been standardized for small children, mainly due to issues related to test safety and tolerance, and to a lack of knowledge on normal bronchoconstrictor response in healthy children.

Most studies of bronchial hyperresponsiveness in small children have been conducted using lung function techniques that require sedation of the patient in order to be able to measure bronchoconstrictor response. Such techniques include the rapid thoracic compression technique (to determine maximum flow at functional residual capacity),[8,9] plethysmography, impulse oscillation, and interrupter resistance.[10-12]

Techniques that do not require sedation of the patient in order to measure bronchoconstrictor response include transcutaneous oxygen pressure measurement[13,14] and the tracheal auscultation method.[15,16] Tracheal auscultation has been validated in older children, and a good correlation has been found with bronchial challenge test results when FEV_1 is used as the response variable.[17-19]

Starting from these premises, we conducted a study based on using the tracheal auscultation method to evaluate methacholine response in healthy children aged under 4 years. Our aim was to establish a normal response pattern for the test and to determine its safety for children of this age.

Population and Methods

Healthy children aged between 6 months and 4 years were recruited for the study in the area of Barcelona, Spain. Children who had been born prematurely or who had a low birth weight, children with cardiovascular and/or lung diseases (including wheezy bronchitis), and children with a family history of allergies and/or passive smoking were excluded from the study. The study received the favorable opinion of the local clinical research ethics committee, and children were only included after parents had been informed and their written consent had been obtained.

A methacholine bronchial provocation test was administered for 2 minutes using the tidal volume breathing technique (in which a continuous flow nebulizer produces an aerosol). Three-milliliter solutions of methacholine chloride (Provocholine, Methapharm Inc, Brantford, Ontario, Canada) diluted in saline were nebulized, then inhaled for 2 minutes by the study subjects using the tidal volume breathing technique.[20] The prerequisites for administering a methacholine challenge test are as follows: no catarrh in the upper airways in the 3 weeks prior to the test, baseline oxygen saturation of 95% or more, normal breath sounds, and no tachypnea. The children were held by a parent and distracted by books or toys suitable for their age while the test was being administered.

The recommendations of the American Thoracic Society (ATS) for the administration of the methacholine bronchial provocation test to children and adults were followed, with some modifications.[21] The ATS protocol, based on doubling the concentration of methacholine between 0.031 mg/mL and 8 mg/mL, results in a total of 10 nebulizations—including the administration of the solution. Given the practical difficulties of using this number of nebulizations for small children, we developed an abbreviated protocol that commenced with a concentration of 0.5 mg/mL, resulting in a total of just 6 nebulizations (Table 1).

The saline and methacholine solutions were administered using a face mask and a nebulizer in the form of a bottle of compressed air operating at a pressure of 15 pounds per square inch (1 bar). A Micromist (Hudson RCI, Temecula, CA, USA) nebulizer was used to give a mass median aerodynamic droplet diameter of between 1 μm and 3.6 μm. The nebulizer was calibrated to calculate the compressed airflow necessary for an output rate of 0.13 mL/min plus or minus 10%; we consequently calculated a flow of 4 L/min to produce a rate falling within this range.

Response to the methacholine challenge was assessed using a modified tracheal auscultation method.[16] Any of the following was rated as a positive response to a specific provocative concentration of methacholine (PCwheeze): tracheal or chest wheezing, a fall of 5% or more in baseline oxygen saturation (SaO_2), or an increase of 50% or more in the baseline respiratory rate.

TABLE 1
The Abbreviated Methacholine Bronchial Challenge Test Protocol Based on the Tidal Volume Breathing Method Compared With the American Thoracic Society (ATS) Methacholine Bronchial Challenge Test Protocol[21]

ATS Protocol	Abbreviated Protocol
Solution	Solution
0.031 mg/mL	–
0.0625 mg/mL	–
0.125 mg/mL	–
0.250 mg/mL	–
0.500 mg/mL	0.500 mg/mL
1 mg/mL	1 mg/mL
2 mg/mL	2 mg/mL
4 mg/mL	4 mg/mL
8 mg/ml	8 mg/mL

Following each nebulization, the trachea and the upper front and lower back parts of the chest were auscultated in 20-second phases, and over the next 3 minutes, respiratory rate, heart rate and SaO_2. were measured at intervals of 1 minute. In the absence of a positive response, successive doses were administered to a maximum concentration of 8 mg/mL. Nebulized salbutamol was administered to children who responded positively.

Statistical Analysis

In order to be able to calculate PCwheeze values for children for whom no response was obtained at a methacholine concentration of 8 mg/mL, it was assumed that a response would have occurred at 16 mg/mL.

Statistical analysis of the data was carried out using the Medcalc program, version 8.1.1.0.

The Kolmogorov–Smirnov test was used to assess the normality of the distribution of the data. The Mann-Whitney nonparametric U test was used for between-group comparisons of quantitative variables, and the Fisher exact text was used to compare qualitative variables. Correlation between quantitative variables was assessed using the Pearson correlation coefficient. Statistical significance was established for a value of P less than .05.

Results

The methacholine challenge test was administered to 16 children (8 girls and 8 boys) aged between 8 and 47 months (mean 23.9 [12.2] months). Demographic details are summarized in Table 2. It was possible to complete the test in all 16 children. Response was negative in 11 children (68.75%) who did not respond to the maximum dose of methacholine (8 mg/mL). There was evidence of a positive response to the maximum concentration of 8 mg/mL in 5 cases (31.25%).

Assuming that a value of 16 mg/mL would have provoked a response in the children with negative challenge responses, the mean PCwheeze value for the group was 13.5 mg/mL (95% confidence interval, 11.17-15.49). The lowest concentration that obtained a response in our patient series was 8 mg/mL; since the methacholine values for the control group were not normally distributed (Kolmogorov–Smirnov test, P=.003), a one-tailed 95% confidence interval was calculated using a nonparametric percentile method. There was no relationship between age and PCwheeze (r=0.01; P=.98), nor was there a difference in response according to sex (P=.6).

Response to the test was considered positive on the basis of tracheobronchial wheezing in 4 cases (accompanied by a fall in SaO_2 in 1 case), and a fall of SaO_2 of more than 5% in 1 case. SaO_2 fell by an average of 3.2% in all 16 children (Table 3), but in no case did it fall below 93%. No significant polypnea or chest indrawing was detected in any subject. The mean increase in respiratory rates was 9.3 breaths/minute (Table 3).

During the test, 7 children developed cough; 4 of these children subsequently responded to a methacholine challenge at a concentration of 8 mg/mL. The cough developed with the concentration of 4 mg/mL, ie, before the positive response was detected. No cough developed

in 1 of the positive responders. Cough developed in 3 children during the final nebulizations, even though no criteria indicated a positive response. Consequently, no relationship could be established between the development of a cough and PCwheeze (P=.12).

Discussion

Our study has demonstrated that it is easy and safe to use tidal volume breathing and modified auscultation for the purpose of assessing bronchial hyperresponsiveness to methacholine in healthy children aged under 4 years. Of the children in our study, 68% had a negative bronchial response, whereas the remainder responded only to methacholine at the maximum concentration of 8 mg/mL. The test is easy to apply, since it requires no active cooperation from children; furthermore, in practice the test has been shown to be feasible, given that all the children in our study were capable of undergoing the test in its entirety.

There are a number of difficulties that can arise in administering this test to small children, such as their inability to understand the test, the discomfort of wearing the face mask necessary for the nebulization, the irritation caused by the methacholine, the possible coughing bouts, the growing sensation of difficulty in breathing prior to a positive response, and the tiredness resulting from the time

TABLE 2
Characteristics Obtained From Medical Records for Study Subjects (n=16)*

Age, mo	23.9 (12.2)
Sex	
Boy	8 (50%)
Girl	8 (50%)
Birth weight, g	3240.6 (465.6)
Gestational age, wk	39.6 (1.8)
Breastfed	14 (87.5%)
Breastfed, mo	5.7 (2.7)
Attendance at day nursery	9 (56%)
Admission age, mo	9.3 (11.1)
No. of brothers/sisters	2.25 (1.84)

*Data are expressed as mean (SD) or as number (percentage).

TABLE 3
Respiratory Rate (RR) and Oxygen Saturation (SaO_2) Changes in Bronchial Challenge Test Responses for the Study Subjects (n=16)*

	RR	SaO_2
Baseline	26.3 (5.6)	97.5 (0.8)
Final	35.6 (7.4)	94.3 (2.3)
Change	+9.3 (6.5)	−3.2 (2.2)
P	.0001	.0001

*Data are expressed as mean (SD).

taken to administer the test. In order to overcome these difficulties it is essential to have the assistance of the child's parents and to create a friendly and playful atmosphere. We used a modified ATS protocol[21] to reduce the duration of the test and ensure completion.[22,23] According to the ATS protocol, 10 inhalations are required; however, small children would be unable to tolerate this number of inhalations, particularly at the later stages. The protocol may thus be abbreviated in any of the following ways: *a)* commence with a higher methacholine concentration, given that positive responses—except for highly hyperresponsive subjects—typically occur above a concentration of 2 mg/mL; *b)* increase successive inhalation doses by more than the usual increments (usually double the previous concentration); and *c)* reduce the administration time of the doses. In our opinion, the risk of severe bronchoconstriction from commencing the test with higher doses or concentrations of methacholine is minimal for patients with mild, stable bronchial asthma who do not require treatment with inhaled steroids; unstable asthma patients, however, should start with the minimum doses and concentrations.[21]

Given that healthy children have a lower theoretical risk of developing bronchoconstriction, we commenced our test with a higher methacholine concentration (0.5 mg/mL), administered following inhalation of an isotonic saline solution. When there was no response we doubled concentrations for the subsequent inhalations, to a total of 6 nebulizations per subject. This approach meant that children were more cooperative and better motivated, and that, in turn, ensured completion of the test. Although some authors propose shortened bronchial provocation tests based on a reduced number of doses of methacholine, this carries the risk of causing severe bronchoconstriction.[24] Our abbreviated protocol led to no severe case of bronchoconstriction nor to any significant reduction in SaO_2.

The sensation of irritation of the airways by methacholine that can lead to coughing bouts may upset children and/or their parents. Nonetheless, coughing may not necessarily indicate an obstructed airway. In our study, 44% of the children developed a cough, irrespective of whether or not bronchoconstriction occurred. Coughing may indeed precede obstruction of the airway, but it may also arise during methacholine nebulization and disappear once inhalation is complete. As pointed out by some authors, coughing may not be a result of bronchoconstriction but may indicate a methacholine dose-response effect that results in irritation of the bronchial and pulmonary cough receptors.[16]

In addition to being practical, the test has been demonstrated to be safe. In order to assess methacholine response, we included 2 additional response criteria besides auscultation, as proposed by Springer et al[16]: a fall of 5% or more in SaO_2 and an increase of 100% or more in the baseline respiratory rate.

No complications were observed in any of the 16 tests performed on these healthy children. The falls in SaO_2 to 93% (2 cases) and the wheezing (4 cases) were brought under control by suspending the administration of methacholine, and salbutamol was used to restore normality. These observations coincide with others in the literature

on the safety of the test.[16,25-27] Only Wilson et al,[28] who conducted a study of children aged 5 years, found that this method could be unsafe; that study was subsequently criticized by a number of other authors, however, for problems of study design that could lead to erroneous conclusions.[19,26]

The reliability and usefulness of the test to assess methacholine response has been validated in studies of children, in which it has been observed that PCwheeze correlates well with spirometer-measured provocative concentration causing a 20% fall in baseline FEV_1 —demonstrating the test's usefulness for evaluating bronchial hyperresponsiveness.[15,17,19,29] The test has also been validated by comparison with phonendoscope auscultation and automated lung sound analysis —methods which would eliminate any possible observer subjectivity. Again, the results from both methods correlated well.[25,30]

In order to study bronchial hyperresponsiveness in small children it is essential to have normal values for healthy children. It has been observed that, since small children have smaller airways, their response to methacholine may be greater, and they may, therefore, experience "normal bronchial hyperresponsiveness." Another important factor to be taken into account is methacholine dosage in relation to body size for small children compared to older children and adults. Use of a standard dose, rather than a dose adapted to weight or measured body surface, means that smaller children receive a proportionally higher dose of methacholine, which may cause them to react more intensely.[31] That said, for the age range studied, we found no differences in response between children of different ages. What we did find was that a third of the healthy children in our series reacted to methacholine at concentrations of 8 mg/mL; consequently, according to our data, a response should only be considered positive if it occurs below this figure. Our results are similar to those of Guirau et al,[32] who proposed a positive response cutoff point of 4 mg/mL on the basis of a study of children aged under 2 years.

Finally, it is important to use a standardized technique that takes into account the type of nebulizer, flow, and above all nebulizer calibration to ensure that the same rate is achieved.[19] The quantity of methacholine inhaled, and consequently, the ultimate response of the individual, may depend on such factors. Our adaptation of the ATS protocol[21] should facilitate the use of a standardized procedure for small children and enable results from different studies to be compared.

We are of the opinion that our modified tracheal auscultation method can be safely and reliably applied to the assessment of bronchial hyperresponsives in unsedated children aged under 4 years. The availability of normal values for this age group would represent a point of departure for the implementation and interpretation of studies of children of this age who have bronchial asthma, wheezy bronchitis, or other diseases such as bronchopulmonary dysplasia. This would, in turn, deepen our understanding of the physiopathologies of these diseases.

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