Bronchial Responsiveness to Methacholine and Adenosine 5′-Monophosphate in Preschool Children With Bronchopulmonary Dysplasia

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Summary. Bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma, but it is also frequently present in children and adults with chronic obstructive lung diseases. Bronchopulmonary dysplasia (BPD) is a chronic lung disease, most commonly developing after mechanical ventilation and oxygen therapy in premature infants. BHR is usually measured by bronchial challenges, using direct or indirect stimuli. The aim of this study was to evaluate BHR to direct and indirect stimuli in young children with BPD. Methacholine and adenosine 5′-monophosphate (AMP) bronchial challenges were performed on preschool children with BPD (n = 19), using a modified auscultation method. The endpoint was defined as the appearance of wheezing and/or oxygen desaturation. The results obtained were then compared with those of asthmatic (n = 25) and control (n = 23) preschool children. A positive response to methacholine (endpoint concentration, ≤8 mg/ml) was observed in 89.5% (17/19) of patients with BPD, but a positive response to AMP (endpoint concentration, ≤200 mg/ml) was observed only in 21.1% (4/19). All patients with asthma responded positively to methacholine, and most (23/25, 92.0%) of them also responded positively to AMP. The majority of controls were unresponsive to both challenges. BHR to methacholine is a frequent finding in preschool-age survivors of BPD, but is usually not accompanied by BHR to AMP. This suggests that most patients with BPD do not have the inflammatory airway response which is characteristic of asthmatic patients. Pediatr Pulmonol. 2006; 41:538–543. © 2006 Wiley-Liss, Inc.

Key words: adenosine 5′-monophosphate; asthma; bronchial hyperresponsiveness; bronchopulmonary dysplasia; methacholine.

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a chronic disease of the lung parenchyma and airways in premature infants, most commonly occurring after mechanical ventilation and oxygen therapy.¹ Although major advances in the care of premature infants have resulted in increased survival, BPD remains a major cause of morbidity and mortality in these patients, affecting approximately 20% of infants with neonatal distress.²

Bronchial hyperresponsiveness (BHR), defined as an exaggerated bronchoconstrictive response of the airways to a variety of stimuli, is considered a hallmark of asthma.³ Survivors of BPD often behave like children with asthma, with recurrent wheezing, shortness of breath, and airflow limitation.⁴ Consistent with this, BHR was repeatedly reported to occur in long-term survivors of BPD, persisting through school age or young adulthood.⁵–⁷ However, information on BHR in young children with BPD is scarce, in part because of the lack of a reliable and sensitive method to evaluate BHR in this age group.

BHR is most commonly evaluated using bronchoconstrictor stimuli such as methacholine or histamine, which act directly at the level of bronchial smooth muscle.⁸ However, BHR can also be assessed using indirect stimuli such as adenosine 5′-monophosphate (AMP), which cause bronchoconstriction by stimulating or enhancing the release of mediators from mast cells.⁹ It was shown that

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BHR to methacholine is present in both asthma and chronic lung diseases, whereas BHR to AMP is present only in asthma. However, to our knowledge, these two types of bronchial challenge have not been compared in patients with BPD.

The aim of this study was to evaluate BHR to methacholine and AMP in young children with BPD, using a modified auscultation method. The results were then compared with those of a group of asthmatic children and a group of healthy controls.

METHODS

Nineteen children with BPD, aged 4–6 years, were included in the study. All children were born prematurely, admitted to the Neonatal Intensive Care Unit, and followed in the Pediatric Pulmonary Clinic at Seoul National University Children’s Hospital. BPD was defined as clinical signs of respiratory distress, chest radiograph abnormalities, and oxygen dependence at 28 days of life. Patients were excluded from the study if they had significant cardiovascular, neurological, or metabolic disease. Out of 28 patients who were followed in the clinic and met inclusion criteria for the study, 9 were not enrolled: 3 due to parental refusal, and 6 because of wheezing and/or oxygen saturation <98% before challenges. Neonatal data were collected from hospital records. The 19 enrolled subjects had a median birth weight of 970 g, a gestational age of 27.4 weeks, duration of artificial ventilation of 45 days, and length of oxygen dependency of 54 days. No significant differences in these variables were observed between participating and nonparticipating subjects (data not shown). Among the 19 patients with BPD, only 4 reported current respiratory symptoms: 2 children had complained of dyspnea with exertion, and 2 children had suffered from continuous coughing for more than 3 weeks during the previous year. None of the patients had received regular medications in a 1-year period prior to the bronchial challenges.

A group of 25 children with asthma, aged 4–6 years, was also studied. These patients had a typical clinical picture of recurrent episodes of asthma symptoms (cough, wheezing, and dyspnea) responsive to antiasthma medications and symptom-free intervals. The symptom pattern was variable, including children with atopic or viral episodic symptoms; no attempt was made to delineate these phenotypes precisely. Nine children had mild intermittent asthma (treated by inhaled β2-agonists), and 16 had mild persistent asthma (treated by inhaled corticosteroids). Patients with a history of near-fatal asthma or major exacerbations necessitating the use of systemic corticosteroids were excluded.

A control group of 23 healthy children was included for comparison. They were selected among children, aged 4–6 years, who had visited our vaccination clinic and had never suffered from wheezing. Their medical history was confirmed by reviewing medical records and by administration of a questionnaire regarding wheezing history. Subjects were excluded from the study if their parents could not answer the questions confidently.

All studied subjects provided blood samples for the determination of total eosinophil counts and serum total IgE, and underwent skin-prick testing for the assessment of atopy. Methacholine and AMP challenges were performed at the same time of day, with an interval of 3–14 days, in a randomized order. All subjects were asked to stop using antihistamine, inhaled bronchodilators, and other medications for 48 hr and inhaled corticosteroids for 7 days before the tests. None of the subjects exhibited any symptoms of an upper respiratory infection in the month preceding the tests.

Methacholine and AMP challenge tests were carried out using a modification of the method described by Springer et al. Fresh solutions of methacholine (0.03–8 mg/ml) and AMP (0.39–200 mg/ml) were made up in a phosphate-buffered solution. All children were required to have normal chest auscultation and an arterial oxygen saturation of ≥98% at baseline. Chest and tracheal auscultation was performed using a regular pediatric stethoscope by two pediatricians, and oxygen saturation was measured by pulse oximetry (Radical®, Masimoet Co., Irvine, CA). Solutions were delivered during quiet tidal breathing via nebulizer (DevBiss 644, DevBiss, Somerset, PA), driven by compressed air at a flow rate of 4.5 l/min; the unit was calibrated to give a mean output of 0.36 ml/min. Inhalations were performed using a face mask and continued for 2 min of tidal breathing, starting with the buffered solution and followed by doubling concentrations of methacholine or AMP every 5 min. After the end of each period of inhalation, the trachea and both lungs were auscultated repeatedly during quiet breathing for about 3 min. The two observers assessed wheezing independently, and wheezing was considered to occur only when their findings coincided. Arterial oxygen saturation was monitored continuously during and after each period of inhalation. The procedure was continued until either an endpoint or maximal concentration was reached. The endpoint was defined as the concentration of methacholine or AMP resulting in audible wheezing and/or a fall in oxygen saturation of at least 5% from baseline (desaturation).
Parents gave written informed consent for their children to participate in the study. The study protocol was approved by the Hospital Ethics Committee.

Statistical Analysis

Data are presented as means ± SD, or as geometric means and a range of 1 SD. Endpoint concentrations were log-transformed before statistical analysis. Subjects were considered to have BHR to methacholine or AMP if they had endpoint concentrations of methacholine ≤8 mg/ml[^13] or of AMP ≤200 mg/ml.[^14] Censored values of 16 mg/ml for methacholine and 400 mg/ml for AMP were awarded to those who did not show a positive response after inhaling the maximal concentration of methacholine (8 mg/ml) or AMP (200 mg/ml). Screening of data for differences between the three groups was performed using one-way analysis of variance (ANOVA) with Tukey’s Honestly Significant Difference (HSD) post hoc test, or the Kruskal-Wallis test with post hoc Mann-Whitney test. Differences in frequencies between two groups were analyzed using the chi-square test. Correlations between variables were calculated using Pearson’s correlation test. A P-value of 0.05 or less was considered significant.

RESULTS

The clinical characteristics and baseline oxygen saturation values for the three study groups are shown in Table 1. There was no significant difference in terms of age or sex ratio between the three groups. The prevalence of atopy, serum total IgE, and blood eosinophil counts were significantly higher in the asthma group than in the other two groups. Oxygen saturation values before methacholine and AMP challenges were both significantly lower in the BPD group than in the other two groups.

Methacholine challenge test results are shown in Figure 1. Seventeen subjects (89.5%) in the BPD group, all 25 subjects in the asthma group, and 4 subjects (17.4%) in the control group responded to methacholine by one or both of the criteria at a concentration of 8 mg/ml or less. In these responsive children, the test was terminated because of wheeze alone in 22 subjects (8 BPD, 12 asthma, and 2 control), both wheeze and desaturation in 10 subjects (4 BPD, 5 asthma, and 1 control), and desaturation alone in 14 subjects (5 BPD, 8 asthma, and 1 control). The frequency of a positive response to methacholine was

![Fig. 1. Endpoint concentrations of methacholine bronchial challenges in three study groups. Horizontal bars represent geometric means. Circles darkened on left half, endpoint concentration attained due to wheeze detection alone; circles darkened on right half, attained due to oxygen desaturation alone; solid circles, attained due to both wheeze detection and oxygen desaturation; open circles, no response.](image)

<table>
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<th>TABLE 1—Clinical Characteristics and Baseline Oxygen Saturation Values of Three Study Groups</th>
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1Means ± SD.
2Geometric mean (range of 1 SD).
P < 0.05 vs. BPD group and control group.
P < 0.01 vs. BPD group and control group.
P < 0.05 vs. asthma group and control group.
significantly higher in the BPD and asthma groups compared to the control group. There was a considerable overlap between the endpoint concentrations of the BPD and asthma groups, and mean values were not significantly different for the BPD (geometric mean (range of 1 SD), 1.24 mg/ml (0.32–4.90)) and asthma groups (1.08 mg/ml (0.28–4.22), $P = 0.30$).

AMP challenge test results are shown in Figure 2. Four in the BPD group, all but 2 subjects in the asthma group, and 2 in the control group responded to AMP by one or both of the criteria at a concentration of 200 mg/ml or less. In these children, the test was terminated because of wheeze alone in 15 subjects (2 BPD, 12 asthma, and 1 control), both wheeze and desaturation in 6 subjects (1 BPD and 5 asthma), and desaturation alone in 8 subjects (1 BPD, 6 asthma, and 1 control). The frequency of BHR to AMP in the asthma group (23/25, 92.0%) was significantly higher than that (4/19, 21.1%) in the BPD group, which was not significantly different from that of the control group (2/23, 8.7%). The asthma group had a significantly lower AMP endpoint concentration (20.35 mg/ml (3.26–127.2)) than the BPD group (231.42 mg/ml (70.32–761.6)).

Of 17 subjects with BPD who had BHR to methacholine, only 3 (17.7%) showed BHR to AMP, whereas the combination of BHR to methacholine and AMP was found in 23 asthma patients (92.0%). Moreover, endpoint concentrations of methacholine and AMP correlated significantly in the asthma group ($r = 0.59$, $P = 0.001$), but not in the BPD group ($r = 0.29$, $P = 0.23$) (data not shown).

There was an inverse correlation between the AMP endpoint concentration and the blood eosinophil count in the asthma group ($r = -0.50$, $P = 0.01$), but not in the BPD group ($r = 0.15$, $P = 0.57$). No correlation was found between the methacholine endpoint concentration and the blood eosinophil count in either the BPD group ($r = 0.18$, $P = 0.43$) or the asthma group ($r = -0.20$, $P = 0.33$) (data not shown).

No relationship was found between the endpoint concentrations of methacholine and birth weight ($r = 0.21$, $P = 0.39$) or gestational age ($r = 0.07$, $P = 0.77$) (data not shown).

**DISCUSSION**

This study is the first to compare methacholine and AMP challenge test results in preschool children with BPD. The majority of these children responded positively to methacholine, but not to AMP. This was in contrast to the findings in asthmatic subjects, most of whom responded positively to both challenges.

Our cohort of BPD patients was derived from those who were followed in a pulmonary clinic. It is conceivable that patients with BPD who have respiratory symptoms and/or require medications tend to be followed, and thus our population might have been biased toward a sicker subgroup of BPD patients. However, this is unlikely, because subjects with relatively severe airflow obstruction, as reflected by wheezing and/or oxygen saturation <98% at baseline condition before challenges, were excluded from the study. In fact, the majority of subjects with recurrent wheezing or other respiratory symptoms during the previous year were excluded according to the above criteria, and most (15/19) of the participating subjects were asymptomatic. With the widespread use of prenatal corticosteroids, exogenous surfactant, high-frequency oscillatory mechanical ventilation, and other modalities, survival of smaller, more premature infants has become a frequent occurrence. The demographic data (gestational age and birth weight) in our BPD patients reflect these advances in neonatal care, and are consistent with reports from other recently studied populations.

In the present study, bronchial challenges were performed using a modified auscultation method that set the two criteria (wheeze detection and oxygen desaturation) to determine the endpoint. For both methacholine and AMP challenges, the incidence of wheeze detection, at the endpoint of a positive challenge, was higher than that of oxygen desaturation. This is consistent with the findings of other studies, and suggests that wheeze detection is a more sensitive indicator of induced bronchoconstriction than oxygen desaturation. We found no significant difference in the incidence of wheezing vs.

**Fig. 2. Endpoint concentrations of adenosine 5-monophosphate (AMP) bronchial challenges in three study groups. Horizontal bars represent geometric means. Circles darkened on left half, endpoint concentration attained due to wheeze detection alone; circles darkened on right half, attained due to oxygen desaturation alone; solid circles, attained due to both wheeze detection and oxygen desaturation; open circles, no response.**
desaturation at the endpoint between the BPD and asthma groups. Subjects were considered to have BHR to methacholine if an endpoint was reached at a methacholine concentration \( \leq 8 \) mg/ml\(^{13,18}\) BHR to methacholine was detected in all asthma subjects, which is comparable to the frequencies reported by two previous studies\(^{13,18}\) that used a similar method. On the other hand, an AMP endpoint concentration of \( \leq 200 \) mg/ml was chosen for BHR to AMP, because this allows asthma patients and healthy controls to be differentiated.\(^{14}\) In fact, all but 2 asthmatic patients in the present study responded positively to AMP \( \leq 200 \) mg/ml. This suggests that the concentration chosen represents an appropriate upper limit for the evaluation of BHR.

In the present study, the vast majority of patients with BPD were hyperreactive to methacholine, but only a minor proportion of these patients were hyperreactive to AMP. Of those subjects hyperreactive to methacholine, BHR to AMP was present in only 3 (17.6%) of 17 patients with BPD, compared to 23 (94%) of 25 asthmatic subjects. This suggests that BHR to methacholine in patients with BPD is usually not accompanied by BHR to AMP, whereas most asthmatic subjects showed positive responses to both methacholine and AMP.

An increased prevalence of BHR to methacholine is consistently found in patients with BPD in infancy,\(^{19}\) school-age children,\(^5,7\) and young adults.\(^6\) The present study is unique in that methacholine responsiveness was evaluated in young children aged 4–6 years. It should be considered that other causes of BHR, such as asthma, might have been overlooked in our BPD patients. However, this is believed unlikely, because most of them were asymptomatic during the preceding year, and the symptoms, when present, were not typical for asthma. Moreover, if that is the case, it is difficult to understand the quite low frequency of BHR to AMP that we found in the BPD patients. Viral infections may cause transient BHR.\(^{20}\) Although none had had symptoms or signs of viral respiratory infections in the 4 weeks preceding the challenges, it is possible that some BPD children with BHR may have had slight infections that were not noticed and that induced transient BHR. However, this is unlikely, because the frequency of BHR was quite low in the control group, for which the precautions against viral infections were identical. The degree of BHR to methacholine is known to be related, to some extent, to the degree of resting airway obstruction as measured by forced expiratory volume in 1 sec.\(^{21}\) Chan et al. also found that BHR in their group of very low birth weight children, aged 7 years, was related to airway caliber.\(^{22}\) Although subjects with audible wheezing or oxygen saturation <98% at baseline were excluded, it is probable that some subjects may have had airflow obstruction that was not screened out by the above criteria. In fact, oxygen saturation values before methacholine challenge tests were significantly lower in the BPD group than in the other two groups. Studies of lung structure from infant autopsies\(^{23}\) indicate that airways of premature infants who require mechanical ventilation have more smooth muscle than those of preterm infants not requiring mechanical ventilation or those of term infants. This hypertrophy of airway smooth muscle might also contribute to the high frequency of BHR to methacholine observed in BPD patients.

In contrast to the similar responses to methacholine, only a minor proportion of BPD patients were hyperreactive to AMP, whereas most of the asthma patients were hyperreactive to AMP. Our results are in accordance with a recent report that unlike children with asthma, school-age survivors of BPD have airflow limitation associated with low exhaled nitric oxide,\(^{24}\) given that BHR to AMP, but not methacholine, significantly correlates with the level of exhaled nitric oxide.\(^{25}\) Two previous studies\(^{10,11}\) showed that BHR to methacholine is present in both asthma and chronic lung diseases (cystic fibrosis, bronchiolitis obliterans, and bronchiectasis), whereas BHR to AMP is present only in asthma. On the basis of our results, BPD could be added to the list of chronic lung diseases that are less responsive to AMP than to methacholine. Of interest, 3 of 4 BPD patients with BHR to AMP had atopy, supporting the hypothesis that atopic status, apart from asthma, is an important determinant for reactivity to AMP.\(^{26}\)

Inflammatory mediator release originating from airway mast cells plays an integral role in the response to inhaled AMP.\(^9\) Clinical studies in asthma patients showed that BHR to AMP reflects an underlying bronchial inflammation more accurately than BHR to methacholine.\(^{27,28}\) This hypothesis is supported by our observation of a positive correlation between blood eosinophil counts and endpoint concentrations of AMP, but not between blood eosinophil counts and endpoint concentrations of methacholine in the asthma group. The poor response to AMP in the BPD group was matched by the absence of an increased blood eosinophil count. No information is available on the nature and pathogenetic relevance of airway inflammation in survivors of BPD beyond infancy. Clarifying this issue would have important and therapeutic implications. In fact, children with BPD are frequently treated empirically with asthma medication,\(^{17}\) although there is no evidence to support this common practice. Our results suggest that most BPD patients do not have an inflammatory airway response which is characteristic of asthmatic subjects.

We made no attempt to include preterm children without a history of BPD as a control group. A number of studies showed that prematurely born children, regardless of BPD, often have BHR.\(^{29}\) In contrast to previous studies,\(^{30}\) birth weight and gestational age were not associated with the degree of methacholine responsiveness, obviously because of the narrow range of gestational ages in our children with BPD. To establish a
relationship between prematurity and bronchial responsiveness, a differently designed study would be needed.

In conclusion, BHR to methacholine is a frequent finding in preschool-age survivors of BPD, but is usually not accompanied by BHR to AMP. This suggests that most patients with BPD do not have the inflammatory airway response which is characteristic of asthmatic patients.

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