

Airway hyper-responsiveness to adenosine 5'-monophosphate in preschool-age children with asthma

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Airway hyper-responsiveness (AHR) to adenosine 5'-monophosphate (AMP) is closely associated with airway inflammation; however, not all asthmatic patients are responsive to it. This study was planned to investigate the predictive factors of AHR to AMP in asthmatic children aged between 3 and 6 yr.

We performed a retrospective analysis of data from 63 asthmatic preschool-age children who were challenged by AMP in our department. All children were characterized by skin-prick tests, serum immunoglobulin E (IgE) levels, peripheral blood eosinophil percentage and bronchial challenge with methacholine (MCH) and AMP. Potential determinants for AHR to AMP were assessed within the group.

AHR to AMP was found in 46% of preschool-age children with asthma, while that of MCH was 93.7%. All children responsive to AMP were also responsive to MCH. The geometric mean provocative concentration of MCH and AMP causing a 15% fall in transcutaneous oxygen tension ($PC_{15}PtcO_2$ MCH and AMP) were 0.55 mg/ml (0.004–9.19) and 10.53 mg/ml (0.59–342.89), respectively. AMP-responsive children did not differ from non-responsive ones with respect to demographic factors, geometric mean $PC_{15}PtcO_2$ MCH and atopic status. The median serum IgE level was significantly higher in AMP-responsive group than the non-responsive ones ($p = 0.011$). The peripheral blood eosinophilia was more frequent among responsive children ($p = 0.019$), and it was found as the only predictive factor for AMP responsiveness in preschool-age children with asthma in logistic regression model (odds ratio: 5.14; 95% CI: 1.23–21.47; $p = 0.025$). AMP responsiveness may be predicted by peripheral blood eosinophilia but not with atopy markers in young children with asthma.

Arzu Bakirtas and Ipek Turktaş

Gazi University, Faculty of Medicine, Department of
Pediatric Allergy and Asthma, Ankara, Turkey

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Arzu Bakirtas, 38. Cadde No: 9/30, 06520
Cukurambar, Ankara, Turkey
Tel.: 00 90 3122025129
Fax: 00 90 3124240040
E-mail: arzub@gazi.edu.tr

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Airway inflammation plays a major role in the pathogenesis of asthma. However, it is often very difficult to obtain data about the pathophysiological events in bronchial mucosa in young children with asthma. Bronchial biopsy and bronchoalveolar lavage are invasive methods and sputum induction is not feasible at this age group. Exhaled nitric oxide measurement, although non-invasive, requires expensive equipment available only in special centers and is difficult to apply especially in children less than 6 yr of

age. Airway hyper-responsiveness (AHR), one of the cardinal features of asthma, is thought to be related to airway inflammation (1–3). AHR may be measured by using either direct or indirect stimuli. Bronchial challenge with a direct stimuli like methacholine (MCH) is a well-known means of screening for AHR. MCH exerts its effect directly on airway smooth muscle. Adenosine 5'-monophosphate (AMP), an indirect stimuli, on the other hand, acts through degranulation of mast cells and release of proinflammatory

mediators. AHR to AMP has been suggested to better correlate with other measures of airway inflammation (4, 5). It has also been demonstrated that AHR to AMP more closely reflects the changes in allergic airway inflammation as a response to asthma treatment with inhaled corticosteroids (6), or after a stay in hypoallergenic environment (7) than MCH. Therefore, AHR to AMP may be used as a tool to evaluate allergic airway inflammation in asthma. If so, defining patients with AMP responsiveness may provide a potential aid in asthma management, especially in young children in whom the clinical picture is more complex with different phenotypes requiring different therapeutic approach (8, 9). Although measuring AHR is not a routine practise in preschool-age children with asthma, it may easily be determined by transcutaneous oxygen monitoring, which requires no sedation or cooperation (10, 11). In this study, we aimed to identify any potential clinical and simple laboratory determinants of AHR to AMP in preschool-age children with asthma that can easily be used in daily practise.

Methods

Database

This was a retrospective analysis of a database of asthmatic children, who were recruited from the outpatient department and had been challenged with AMP for inclusion in clinical trials. Initially, we had recruited 91 children aged between 3 and 6 yr, with recurrent wheezing and cough for possible inclusion in the clinical trials from the outpatient department. Twenty-two of them had risk factors for transient early wheezing, such as a history of premature delivery, low birth weight or maternal smoking during pregnancy, and therefore were not included to avoid a misclassification in asthma group. One child with bronchiectasis, four with gastroesophageal reflux and one with cystic fibrosis were also excluded because of chronic lung disease. The remaining 63 young preschool-age children with recurrent wheezing responded favorably to a treatment with inhaled corticosteroids and/or inhaled short-acting bronchodilators and were diagnosed as asthmatic. Bronchial challenge with AMP was performed only in those who were diagnosed with asthma. At the time of inclusion in clinical trials, a standard file consisting of a detailed entry questionnaire, including age at onset of disease, duration and severity of respiratory symptoms, trigger for symptoms, presence of other associated atopic diseases, type and dose of

previous medical treatment, family history and home conditions, was filled out for every child diagnosed as asthmatic. These children were also objectively assessed by physical examination, skin-prick tests (SPT), serum immunoglobulin E (IgE) levels and serum eosinophil counts. All data in patient files were regularly entered in the database program. The clinical trials that included the children in our retrospective analysis had been reviewed and approved by the Medical Ethics Committee and parental informed consent had been obtained at the time the children were included in the trial.

Bronchial challenge tests

Solutions of MCH (acetyl- β -methylcholine chloride) and AMP (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were prepared daily from a stock solution in doubling concentrations from 0.031 to 16 mg/ml and 0.39 mg/ml to 400 mg/ml, respectively. They were administered at room temperature as aerosols generated from a starting volume of 2 ml. Bronchoconstrictor agents were delivered by a DeVilbiss 646 nebulizer (DeVilbiss Co, Somerset, PA, USA) attached to a compressor, which produced an output of 0.13 ml/s (Pulmo Aide compressor by DeVilbiss, USA). A shortened validated protocol, suitable for use in preschool-age children was used for bronchial challenges (12). According to this protocol, preschool-age children were monitored by a transcutaneous oxygen monitor (TCO₂M transcutaneous monitor; Novamatrix Medical Systems Inc., Wallingford, USA) throughout the challenge procedure. The temperature of the sensor was 44°C and it was placed on anterior chest wall below the shoulder area as recommended by the company for pediatric use. Transcutaneous oxygen tension (PtcO₂) was stable in about 20–30 min. The baseline PtcO₂ was recorded. MCH or AMP were inhaled after a diluent step (normal saline) using 2-min tidal breathing method through a face mask (13). Quadrupling concentrations were administered at 5-min intervals until the PtcO₂ had fallen 10% from the baseline value. Doses of bronchoconstrictor agents were doubled thereafter until at least a 15% decrease in PtcO₂ was recorded from baseline, or until the maximum concentration was given. At each concentration step, the variations in PtcO₂ were followed for 3 min and the lowest value of PtcO₂ was recorded manually. Provocative concentration of MCH and AMP causing a 15% fall in transcutaneous oxygen tension (PC₁₅PtcO₂) were calculated from the log dose–response curve by linear

interpolation. For safety, children were also monitored by a pulse-oxymeter (Nellcor Puritan Bennett, Inc. Pleasanton, CA, USA) and auscultated over trachea and chest at each concentration step. Short-acting bronchodilators were not allowed within 8 h before the test. Bronchial challenges were done only if the child was free of asthma symptoms for at least 2 months. Bronchial challenges were postponed for at least 6 wk in children with respiratory tract infection. MCH and AMP challenges were done within a week in the same patient. All preschool-age children who had been challenged by AMP were always first screened by MCH challenge. All challenges were performed by one of the authors (A. Bakirtas) between 08:30 and 10:30 am. Since bronchial challenge is done in a sitting position and is relatively long for this young age group, a cartoon movie was shown by a VCD player throughout the procedure. A $PC_{15}PtcO_2MCH < 16$ mg/ml was considered the cut-off value for airway responsiveness to MCH (14). Although there has been no epidemiologic data for provocative concentrate of AMP causing a 20% decrease in forced expiratory volume in 1 second ($PC_{20}AMP$), a cut-off value of ≤ 200 mg/ml is generally accepted as airway responsiveness to AMP in both children (15) and adult (16–18) patients with asthma. Therefore, we also applied this threshold to define our patients as responsive ($PC_{15}PtcO_2AMP \leq 200$ mg/ml) and non-responsive ($PC_{15}PtcO_2AMP > 200$ mg/ml) to AMP.

Skin-prick test

SPT were performed on the back in preschool-age children by the use of standard skin-prick method. The tests was carried out with the standardized allergen extracts against the following eight most common aeroallergens: house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), grass mix, weed mix, cereal mix, and tree mix, cockroach, *Alternaria*, *Cladosporium*, *Aspergillus*, cat and dog dander (Stallergens, France). Histamine hydrochloride (10 mg/ml) and allergen diluents were used as positive and negative controls, respectively. A mean wheel diameter, at least 3 mm greater than that of the negative control, was considered positive. Any child with such a response to one or more allergens was defined as atopic.

IgE and eosinophil measurements

Total serum IgE levels were determined using a chemiluminescence automatic immunoassay (Beckman Coulter, Fullerton, CA, USA) and

expressed in IU/ml. Eosinophil counts were obtained from blood smears by routine methods. A differential of 300 white blood cells on peripheral blood smears was used to determine percentage of eosinophils. A cut-off value of 4% was used to define peripheral blood eosinophilia (19, 20).

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 11.0. The distribution of the parameters was inspected by normal probability plots. Kolmogorov–Smirnow test was used to formally evaluate the normality of distributions. $PC_{15}PtcO_2$ values were logarithmically transformed to normalize data. Between-group comparisons were done by the Mann–Whitney test for the duration of asthma, age at first wheezing episode, serum IgE level and peripheral blood eosinophil count; the unpaired student's *t*-test for age, birth weight and logarithmically transformed $PC_{15}PtcO_2$ MCH values. Categorical variables were analyzed with the chi-square or Fisher's exact test, as appropriate, to find the differences in proportions. Variables were kept in the logistic model if the *p*-value for their independent effect was < 0.05 .

Logistic regression analysis was then conducted to identify independent predictive factors for AMP responsiveness. Both statistically significant and clinically important variables were included in the regression model with adjustment for age and sex. A *p* value of < 0.05 was accepted as the minimum level of statistical significance.

Results

We identified 63 preschool-age children with asthma that were challenged by AMP in our database. Demographic data are shown in Table 1. There were more boys (58.7%) than girls (41.3%). The age of children ranged between 3 and 6 yr. Eleven children (17.5%) had a first-degree relative with asthma. All children had asthma symptoms for at least 2 yr. A total of 21 (33.3%) children were atopic, as demonstrated by positive SPT to at least one inhalant allergen. None of the children were taking inhaled steroids, leukotriene receptor antagonists or anti-histamine drugs within 1 month of bronchial challenges. All but four children did not respond to MCH even at the highest concentration (16 mg/ml). Of the 63 asthmatic preschool-age children challenged by AMP, 29 (46%) were responsive to AMP at or below 200 mg/ml. Three children had a

Table 1. Demographic and laboratory data of preschool-age children with asthma

Number of patients	63
Boy/girl	37/26
Age at the time of challenge, months (mean $\pm$ s.d.)	52.74 $\pm$ 9.51
Birth weight, gram (mean $\pm$ s.d.)	3,363.49 $\pm$ 462.26
Median duration of asthma, months (range)	33 (24–64)
Median age at first wheezing episode, months (range)	18 (2–48)
Smoking at home (%)	27 (42.9)
Family history of asthma (%)	11 (17.5)
Skin-prick test positivity (%)	21 (33.3)
Atopic dermatitis (%)	7 (11.1)
Allergic rhinoconjunctivitis (%)	17 (27.4)
Median serum IgE level, IU/ml (range)	42.18 (2.32–1,548.00)
Peripheral blood eosinophilia (%)	15 (23.8)
Methacholine responsiveness (%)	59 (93.7)
AMP responsiveness (%)	29 (46)

IgE, immunoglobulin E; AMP, adenosine 5'-monophosphate.

PC₁₅PtcO₂AMP value between 201 and 400 mg/ml. The geometric mean of PC₁₅PtcO₂MCH and AMP values of children included in the analysis were 0.55 mg/ml (0.004–9.19) and 10.53 mg/ml (0.59–342.89), respectively. All children responsive to AMP were also responsive to MCH. AMP-responsive and non-responsive children did not differ with respect to age, gender, birth weight, duration of asthma, age at first wheezing episode, smoking at home, family history of asthma, atopic sensitization on SPT, atopic dermatitis and allergic rhinitis ($p > 0.05$) (Table 2). Among the AMP-responsive group, 13 children (44.8%) were atopic, while 16 (55.2%) were non-atopic. However, in AMP non-responsive group there were 8 atopic and 26 non-atopic

Table 3. Multivariate linear regression: potential predictor factors for airway hyper-responsiveness to adenosine 5'-monophosphate in preschool-age children with asthma

Independent variable	Odds ratio	95% CI	p-value
Atopic sensitization by SPT	2.117	0.543–8.261	0.280
Serum total IgE level	1.005	0.997–1.013	0.186
Peripheral blood eosinophilia	5.144	1.233–21.475	0.025

SPT, skin-prick test; IgE, immunoglobulin E.

Peripheral blood eosinophilia: $>4\%$ eosinophil on peripheral blood smears.

children (23.5% and 76.5%, respectively). The median serum IgE level was significantly higher in AMP-responsive group than the non-responsive one ($p = 0.011$). There was also a significant difference between the AMP-responsive and non-responsive children considering peripheral blood eosinophilia ($>4\%$) (37.9% and 11.8%, respectively; $p = 0.019$). Geometric mean of PC₁₅PtcO₂MCH values were similar in the two groups ($p = 0.177$).

The serum total IgE level, peripheral blood eosinophilia and atopic sensitization by SPT were included as independent variables in multivariate logistic regression model to identify the determinants of AMP responsiveness. Peripheral blood eosinophilia was found as the only predictive factor for AMP responsiveness in preschool-age children with asthma after adjustment for age and gender (odds ratio: 5.14; 95% CI: 1.23–2.47; $p = 0.025$) (Table 3).

Discussion

The results of this analysis showed that almost half of the 63 preschool-age children with

Table 2. Comparison of preschool-age children with asthma according to their response to bronchial challenge with AMP

	PC ₁₅ PtcO ₂ AMP (mg/ml)		p
	≤ 200	>200	
Boys (%)	16 (55.2)	21 (61.8)	0.596
Age at the time of challenge, month (mean $\pm$ s.d.)	54.13 $\pm$ 10.40	51.55 $\pm$ 8.66	0.287
Birth weight, gram (mean $\pm$ s.d.)	3,361.72 $\pm$ 520.00	3,365 $\pm$ 414.75	0.978
Median duration of asthma, months (range)	30 (24–56)	33.5 (24–64)	0.694
Median age at first wheezing, months (range)	18 (3–48)	12 (2–48)	0.203
Smoking at home (%)	15 (51.7)	12 (35.3)	0.189
Family history of asthma (%)	7 (24.1)	4 (11.8)	0.197
Skin-prick test positivity (%)	13 (44.8)	8 (23.5)	0.074
Atopic dermatitis (%)	4 (13.8)	3 (8.8)	0.694
Allergic rhinoconjunctivitis (%)	9 (31)	8 (24.2)	0.550
Median serum total IgE level, IU/mL (range)	56.4 (3.67–1548)	28.2 (2.32–3.99)	0.011
Peripheral blood eosinophilia (%)	11 (37.9)	4 (11.7)	0.019
Geometric mean of PC ₁₅ PtcO ₂ MCH (mg/ml) (range)	0.42 (0.05–8.77)	0.72 (0.04–9.19)	0.177

IgE, immunoglobulin E; AMP, adenosine 5'-monophosphate; PC₁₅PtcO₂MCH, provocative concentration of methacholine causing a 15% fall in transcutaneous oxygen tension; PC₁₅PtcO₂AMP, provocative concentration of AMP causing a 15% fall in transcutaneous oxygen tension.

well-documented asthma have airway responsiveness to AMP. Among them, 59 were responsive to MCH (93.7%), while 29 were responsive to AMP (46%). The AMP-responsive children had significantly higher serum IgE level and peripheral blood eosinophilia than the non-responsive ones. Neither the SPT positivity nor the severity of MCH responsiveness predicted the response to AMP challenge in young children with asthma. In multivariate logistic regression analysis only peripheral blood eosinophilia was found to predict the responsive group.

Airway responsiveness to AMP has been shown to be more specific for asthma diagnosis both in preschool-age (21) and school-age children (22). It has even been used in some clinics as the bronchial challenge of choice in young children who were referred for a possible diagnosis of asthma with a chief complaint of unexplained cough without wheezing (23, 24). In contrast to its popularity, there is very limited data about the rate of AHR to AMP in childhood asthma (21, 22, 25). Among them, the one by Avital et al. (21) provides information about this issue for the preschool-age children with asthma. In this study, the authors showed that all of the 39 young children with a mean age of 4.3 yr, who met the American Thoracic Society's diagnostic criteria for asthma, responded to AMP below a concentration of 400 mg/ml. In our analysis, 32 children (50.7%) responded to AMP at ≤ 400 mg/ml and 29 children (46%) responded at ≤ 200 mg/ml. This ratio of AMP responsiveness is much lower than the one that was reported by Avital et al. (21). An interference of transient early wheezers with our results seems unplausible as they were not included in the analysis. One could not propose an effect of therapy on the rate of AMP responsiveness, because none of the children received inhaled steroids, anti-histamines or leukotriene receptor antagonists within a month of bronchial challenge. We cannot explain this difference with the different methods used in the two studies, because in addition to PtcO₂, we also monitored our patients by auscultation and pulse oxymeter for safety reasons, and none of the children challenged had wheezing either on auscultation or desaturation (defined as a fall in oxygen saturation) of $\geq 5\%$ from baseline before a 15% change in PtcO₂. All the children included in our analysis had stable asthma for at least 2 months at the time of challenge procedure. Therefore, we estimate that the differences in the rate of AMP responsiveness between Avital et al.'s study (21) and ours is more likely to be explained by the differences in the population included in them.

Our study is the first report on factors related to AHR to AMP in preschool-age children with asthma. We investigated potential demographic, clinical and laboratory factors that might be associated with AHR to AMP. There is no study reporting the relationship between demographic factors, such as age, gender, birth weight, duration of asthma and family history of allergic diseases and AHR to AMP in either childhood or adulthood. However, one study in school-age children with asthma compared the demographic characteristics of those who were responsive to MCH with the non-responsive ones after the withdrawal of inhaled corticosteroid therapy (26). In this study, the authors did not demonstrate any difference between the two groups considering the demographic characteristics. We did not find any putative demographic factors, such as birth weight, gender, age, duration of asthma, age at first wheezing episode, smoking at home and family history of asthma for AMP responsiveness in young children with asthma, either.

Studies in adult patients with asthma suggested that atopy is closely associated with AMP responsiveness (27, 28). We investigated the influence of atopy markers, such as SPT positivity, serum total IgE, atopic dermatitis and allergic rhinoconjunctivitis on AHR to AMP in our young patients with asthma. Only serum total IgE but not SPT positivity, atopic dermatitis or allergic rhinitis were found significantly higher in AMP-responsive group compared with the non-responsive ones in univariate analysis. However, we failed to identify any atopy-related factor discriminating AMP-responsive children from the others in the young age group by multivariate logistic regression analysis. On the other hand, we recently reported that atopy is the only predictor of AMP responsiveness in school-age children with asthma (29). Therefore, we also think as Kono et al., who suggested that the relation between total serum IgE and AHR may be age-dependent; in other words, it is stronger in older children compared with young ones below 5 yr of age (30). An interesting finding in our analysis is that 55.2% of AMP-responsive children were non-atopic by SPT. However, we think that two potential sources of bias might have confounded this result. One is the sample size of our analysis that might have limited power to assess the independent effect of atopy on airway responsiveness to AMP. The other is the definition of atopy in the analysis, which was at least one positive response on SPT. It is well known that SPT are specific, but not very sensitive for a definition of atopy in young children because of

low levels of skin reactivity (31). As a result, we might have underestimated the atopic sensitization in the preschool-age children with asthma. On the other hand, non-atopic adult patients with asthma also demonstrate airway responsiveness to AMP suggesting that mast cell activation does not solely depend on the presence of atopy, but is also a part of the asthmatic inflammatory process (32). The AHR to AMP may thus not be driven predominantly by atopic sensitization in young children with asthma.

Eosinophilic inflammation, an important feature of asthma, was shown to be present together with early signs of airway remodeling even before a diagnosis of asthma would be made by clinical symptoms in children (33). Peripheral blood eosinophilia has long been recognized as one of the non-invasive eosinophilic inflammatory markers (19) and was defined as the minor criterion in 'asthma predictive index' (20). In our analysis, we also used peripheral blood eosinophilia as inflammatory marker and found it as the only predictor of AMP responsiveness in preschool-age children with asthma. A positive association between peripheral blood eosinophil count and AHR to AMP was also shown in studies with adult patients (5, 27). de Meer et al. measured a risk estimate as prevalence ratio and reported that adults with a blood eosinophil count $\geq 275/\text{mm}^3$ have a 3.57-fold increased risk of AMP responsiveness (27). Our results are consistent with these studies and suggest that AMP responsiveness in asthmatic preschool-age children may be related to eosinophilic inflammation. Although we are aware of the limitations of a retrospective study, such as unrecognized group differences and bias, we feel that our results may be used as preliminary data to define the ultimate prospective study.

In conclusion, AMP responsiveness was present in almost half of the preschool-age children with asthma and was predicted by peripheral blood eosinophilia, but not with atopy markers in the young age group in contrast to adults and school-age children with asthma. Prospective studies are still required in order to clarify the role of AMP responsiveness in determining the clinical phenotypes and management of asthma in young children.

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