Bronchial reactivity and intracellular magnesium: a possible mechanism for the bronchodilating effects of magnesium in asthma

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ABSTRACT

1. Increased bronchial smooth muscle contractility with consequent bronchial hyperreactivity are characteristic physiopathological events of asthma. Since magnesium intervenes in calcium transport mechanisms and intracellular phosphorylation reactions, it constitutes an important determinant of the contraction/relaxation state of bronchial smooth muscle. In the present study we investigated the relationship between bronchial reactivity, assessed by methacholine-provocation test, and magnesium concentrations both at extracellular and intracellular levels measured by spectrophotometry. Twenty-two patients with mild-to-moderate asthma and 38 non-asthmatic subjects with allergic rhinitis (24 allergic to Parietaria pollen and 14 allergic to Grass pollen) were recruited to the study. Exclusion criteria included renal failure, hepatic diseases, heart failure and arterial hypertension.

2. The salient finding of our study is that there is a strong positive correlation between bronchial reactivity and the level of intracellular magnesium ($r = 0.72$, $P < 0.0001$), both when the groups are analysed separately or together. Intracellular magnesium concentrations in the group of patients with asthma were significantly lower ($1.8 \pm 0.01$ mmol/l; $n = 22$) when compared with levels in rhinitis subjects allergic to Parietaria ($1.9 \pm 0.01$ mmol/l; $n = 24$, $P < 0.05$), and with levels in rhinitis subjects allergic to Grass pollen ($2.0 \pm 0.03$ mmol/l; $n = 14$, $P < 0.05$). Serum levels of the ion were similar in all groups.

3. We conclude that the level of intracellular magnesium may be an important determinant of bronchial hyperreactivity, as supported by the significant positive correlation between these two parameters in allergic patients with known bronchial hyperresponsiveness. This finding, in addition to reports of the bronchodilating effects of magnesium administration in patients with asthma, confirms the proposed role of this ion in the pathogenesis and treatment of asthma.

INTRODUCTION

Magnesium is closely involved in numerous important biochemical reactions in the body, particularly those processes that entail the formation and utilization of ATP [1]. As a cofactor of over 300 intracellular enzymic reactions utilizing high-energy phosphate bonds, magnesium has been implicated in smooth muscle contraction.

Key words: asthma, bronchial hyperreactivity, bronchial smooth muscle, intracellular ions, magnesium.

Abbreviations: $\text{FEV}_{1.0}$ forced expiratory volume in 1.0 s; $\text{PC}$, provocative concentration.

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[1,2]. In fact, magnesium is a cation with modulatory effects on the contractile state of smooth muscle cells in various tissues: hypomagnesaemia leads to contraction [3,4] and hypermagnesaemia leads to relaxation [5,6].

Magnesium has been shown to relax bronchial smooth muscle in vitro [2] and to bronchodilate asthmatic airways in vivo [7,8]. Potential mechanisms for the direct relaxing effects of magnesium on bronchial smooth muscle include calcium channel blocking properties [9–11], inhibition of cholinergic neuromuscular transmission with decreased sensibility to the depolarizing action of acetylcholine [12,13], stabilization of mast cells and T-lymphocytes [14,15], and stimulation of nitric oxide [16] and prostacyclin [17].

On the basis of the critical role of magnesium in the regulation of bronchial smooth muscle cell contractility via effects on calcium transport activation, calcium cellular content, and phosphorylation/dephosphorylation intracellular reactions, it has been proposed that the intracellular magnesium level may determine the excitability of these cells [1,2]. Magnesium deficiency, either absolute or relative, may then lead to an increased excitability of bronchial smooth muscle with a consequent bronchoconstriction [13]. In accordance with this hypothesis, Britton et al. [18] demonstrated that dietary magnesium intake is independently related to lung function and to the occurrence of airway hyperreactivity, suggesting that a low magnesium intake may be involved in the aetiology of asthma.

Additionally, current clinical data have demonstrated a bronchial myorelaxant action of magnesium sulphate administration during bronchospasm [7,8,19–22]. The cellular mechanisms responsible for this bronchodilation remain to be elucidated but may involve bronchial smooth muscle relaxation similar to magnesium’s effects on vascular smooth muscle via calcium antagonism [9–11] or some other mechanism [12–16].

We have recently demonstrated that subjects with rhinitis allergic to Parietaria pollen may have methacholine-provocation test values in the asthmatic range, and are at higher risk of developing non-specific bronchial hyperresponsiveness when compared with subjects allergic to Grass pollen who exhibit methacholine test values in the normal range [23].

The purpose of this study was to investigate the relationship between non-specific bronchial reactivity and the level of magnesium, both at an intracellular level and in the serum, in three groups of subjects: (a) asthmatic patients with known bronchial hyperresponsiveness; (b) patients with rhinitis allergic to Parietaria pollen, who may have normal or compromised bronchial responsiveness; and (c) patients with rhinitis allergic to Grass pollen, who have normal bronchial responsiveness to methacholine [23].

**SUBJECTS AND METHODS**

**Subjects**

We studied a total of 60 subjects, selected from the ambulatory Allergy Clinic of the Istituto di Medicina Interna e Geriatria from the University of Palermo. Twenty-two subjects (mean age, 33.7 ± 2.0 years; male/female, 4/18) had a confirmed diagnosis of mild-to-moderate asthma according to the American Thoracic Society’s definitions of asthma [24]. These subjects had a baseline forced expiratory volume in 1.0 s (FEV\(_{1.0}\)) of at least 80% of the predicted value and a provocative concentration of inhaled methacholine causing a 20% fall in FEV\(_{1.0}\) (PC\(_{20}\)) ≤ 4 mg/ml. All patients with asthma were allergic to Parietaria pollen, which is an Urticacea, and the most important rhinitis-provoking plant in Southern Europe [25]. In addition there were 24 subjects with rhinitis allergic to Parietaria pollen (mean age, 33.9 ± 1.9 years; male/female, 7/17), and 14 subjects with rhinitis allergic to Grass pollen (mean age, 27.7 ± 1.4 years; male/female, 3/11). The clinical characteristics of the three groups are reported in Table 1.

The protocol was approved by the Ethics Committee of the Istituto di Medicina Interna e Geriatria and was conducted according to the guidelines of the Declaration of Helsinki (1989). Informed consent was obtained from each subject. Patients with renal failure, hepatic diseases, heart failure or arterial hypertension were excluded from the study. None of the patients was taking steroids, calcium or diuretics. Bronchodilating therapy and antihistamines were discontinued 72 h before the provocation test with methacholine and blood sampling.

**Lung function and non-specific bronchial reactivity measurements**

Bronchial hyperresponsiveness was evaluated with the methacholine-stimulation test described by Chai et al. [26] and used before in our laboratory [23,27]. FEV\(_{1.0}\) was measured with a Gould 2400 automated system, taking the highest of three successive measurements, provided that the difference between measurements was within 100 ml. All patients with asthma had a basal FEV\(_{1.0}\) of at least 80% of the theoretical value. After baseline determination of FEV\(_{1.0}\), subjects inhaled five puffs of saline solution; this diluent value was considered as the control, and if the FEV\(_{1.0}\) variations were within 10% of baseline, the patients were entered into the study. Subjects were then asked to inhale increasing concentrations of methacholine, ranging from 16 to 5120 µg/ml, administered with a MEFAR nebulizer (Markos, Monza, Italy). FEV\(_{1.0}\) was measured 90 s after each concentration step. The provocation test was terminated when FEV\(_{1.0}\) fell by at least 20% with respect to the baseline value (PC\(_{20}\) = FEV\(_{1.0}\)) or when a maximum cumulative dose had been given. Log-cumulative methacholine doses were
Table 1  Clinical data of patient groups studied

Values are means ± S.E.M. Mgi, intracellular magnesium; Mge, serum magnesium; NS, not significant.

<table>
<thead>
<tr>
<th>Patient group . . .</th>
<th>Asthma</th>
<th>Rhinitis</th>
<th>Parietaria</th>
<th>Grass</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>33.7 ± 2.0</td>
<td>33.9 ± 2.0</td>
<td>27.7 ± 1.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>FEV₁,₀ (L)</td>
<td>2.82 ± 0.1</td>
<td>3.05 ± 0.1</td>
<td>3.32 ± 0.1</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>FEV₁,₀ (%)</td>
<td>95.6 ± 2.4</td>
<td>101.8 ± 2.4</td>
<td>98.7 ± 1.5</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Methacholine (mg/ml)</td>
<td>260.6 ± 40</td>
<td>1470.8 ± 73</td>
<td>2028.6 ± 56.9</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Mgi (mmol/l)</td>
<td>1.8 ± 0.01</td>
<td>1.9 ± 0.01</td>
<td>2.028 ± 0.04</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Mge (mmol/l)</td>
<td>0.9 ± 0.03</td>
<td>0.9 ± 0.02</td>
<td>1.016 ± 0.01</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Magnesium measurements

After overnight fasting a blood sample was collected from each subject into tubes containing heparin. Erythrocyte intracellular magnesium levels were determined according to the method described by Paolisso et al. [28]. Briefly, erythrocytes were isolated by centrifugation (5000 r.p.m. for 15 min) and the precipitate was washed three times with an isotonic saline solution (150 mmol/l NaCl). Cells were counted to normalize samples, and were then lysed osmotically by the addition of deionized water, allowing the solution to stand for 30 min. After this the solution was centrifuged and the supernatant used for magnesium determinations. Serum and erythrocyte magnesium concentrations were measured by atomic absorption spectrophotometry (Cobas DP25). All assays were performed in duplicate.

Statistical analysis

The results are expressed as means ± S.E.M. One-way analysis of variance with appropriate post hoc test for multiple group comparisons was performed. To evaluate the relationship between specific bronchial reactivity, intracellular and extracellular magnesium concentrations, we calculated Spearman’s correlation coefficients, utilizing SPSS 6.0 software. A P value ≤ 0.05 was considered statistically significant.

RESULTS

Basal FEV₁,₀, percentage FEV₁,₀ and methacholine concentrations (PC₂₀ – FEV₁,₀) for both asthmatic and rhinitic subjects are shown in Table 1.

Intracellular magnesium levels were significantly lower in patients with asthma (1.8 ± 0.01 mmol/l) compared with the levels in patients with allergic rhinitis Parietaria-sensitive (1.9 ± 0.01 mmol/l; P < 0.05) and in patients with Grass-sensitive allergic rhinitis (2.028 ± 0.04 mmol/l; P < 0.05) (Table 1 and Figure 1). Serum magnesium levels were comparable in all groups (Table 1) and within the normal range [1].

Looking at the relationship among the different variables, we found a highly significant correlation between intra-erythrocytic magnesium and the response to inhaled methacholine (PC₂₀ – FEV₁,₀) in the three groups of subjects studied when analysed together (r = 72, P < 0.0001) or separately (asthma: r = 0.80, P < 0.0001; Parietaria-sensitive rhinitis: r = 42.2, P < 0.05; Grass-sensitive rhinitis: r = 83.6, P = 0.0002) (Figure 2). The relation between serum magnesium and PC₂₀ – FEV₁,₀ was not statistically significant for all groups (P > 0.05). FEV₁,₀ and intracellular magnesium were not correlated in subjects with asthma (P > 0.05).

DISCUSSION

Magnesium’s role in asthma was first suggested by anecdotal reports of favourable effects of magnesium sulphate administration in acute exacerbations of asthma.
over 50 years ago [29,30]. Later, Durlach [14] reported a reduction in serum magnesium levels during the acute attack of asthma. More recently, the possible role of magnesium in the pathogenesis of bronchial constriction as well as in its treatment has regained considerable attention, particularly because of several reports confirming positive results of magnesium administration in acute asthma attacks [7,8,19–22,31], although some studies have reported negative results [32–37]. Even in the absence of an acute exacerbation, the functional pulmonary tests have been shown to improve with the administration of intravenous magnesium [38], and the action of magnesium appears to be additive to the bronchodilating effect of the anti-asthmatic drugs terbutaline [39] and salbutamol [40]. However, the mechanism of these effects has not yet been clarified.

The processes of contraction and relaxation of the myofibrillar proteins in bronchial smooth muscle cells are the result of phosphorylation and dephosphorylation reactions regulated by specific enzymes and by the intracellular content of calcium [1,2]. Since bronchial smooth muscle cells possess only one membrane system and a scarce sarcoplasmic reticulum, calcium transport across the cellular membrane is the most important regulator of intracellular calcium content, which is mainly attained by the action of a calcium/magnesium-dependent membrane ATPase, and by voltage and receptor-operated calcium channels [1].

The enzymes that regulate phosphorylation/dephosphorylation reactions are from two classes: protein kinases and phosphoprotein phosphatases. The first group includes myosin kinases which phosphorylate myosin chains, and the second group includes myosin phosphatases which dephosphorylate the light chains of myosin. Myosin phosphatases are calcium-dependent enzymes, whereas myosin kinases are magnesium-dependent enzymes [1,3]. Since magnesium is involved in calcium transport across the cellular membrane [9–11], which determines intracellular calcium content, both types of enzymes are directly or indirectly influenced by intracellular magnesium levels [1,3]. Such effects of magnesium would be expected to result in relaxation of bronchial smooth muscle and reduction of the airway reactivity to inhaled bronchoconstrictor agents.

Our results demonstrate that in cells from patients with asthma and from patients with rhinitis, the levels of intracellular magnesium are directly and strongly related in a continuous manner to the reactivity of the bronchial airway, measured as the concentration of inhaled methacholine causing a 20% fall in FEV$_{1.0}$ (PC$_{20}$–FEV$_{1.0}$). Erythrocytes provide a feasible experimental model that has been demonstrated to allow repeatable and accurate measurements of intracellular ions [28,41,42]. In agreement with our findings are reports demonstrating that magnesium administration specifically reduces methacholine- and histamine-induced bronchoconstriction in patients with asthma [43,44]. This relation may also help explain the favourable action of magnesium supplementation during acute asthma attacks. Although the intracellular and serum levels of magnesium in all the patients were in the normal range, we found a significantly lower level of intracellular magnesium in patients with asthma when compared with cells obtained from the non-asthmatic subjects with allergic rhinitis. Interestingly, Parietaria-sensitive patients, who have been demonstrated to be at higher risk of non-specific bronchial hyperreactivity [23], had an intermediate magnesium level compared with those of asthmatic and those of Grass-sensitive patients, who have normal bronchial responsiveness [23]. This parallels the continuous relation of PC$_{20}$–FEV$_{1.0}$ and intracellular magnesium in the three groups of subjects. Thus we cannot exclude the possibility that a relative intracellular magnesium deficit may contribute to the bronchial hyperreactivity characteristic of patients with asthma. As such, a relative intracellular magnesium deficiency in asthma may favour the movement of calcium to inside the smooth muscle cell, leading to a potentiation of myosin phosphorylation and rendering the cell more contractile. On the other hand, magnesium may determine the sensitivity of the bronchial smooth muscle to acetylcholine. An excess of magnesium may decrease the depolarizing action of acetylcholine, resulting in a depressed excitability of the bronchial smooth muscle cells.

Since magnesium is predominantly an intracellular ion, and there is a concentration gradient between the extracellular and intra-cellular compartments [1], serum levels of magnesium may not reflect intracellular magnesium content. The apparent lack of correlation between levels
of serum and tissue magnesium makes it difficult to interpret their relationship. Further, considering only magnesium levels in the serum, it is possible to overlook an intracellular hypomagnesaemia [3], which may correspond to a true reduction in total body stores of the ion. In fact, and in agreement with our results regarding serum magnesium levels, Falkner et al. [45] found no difference in this parameter in patients with asthma during acute exacerbation compared with a non-asthmatic population. They concluded that serum magnesium measurements are not clinically useful for predicting the severity of the attack, nor are they predictive of the response to magnesium infusion [45].

Regarding the role of magnesium as a determinant of bronchial hyperreactivity, an interesting study by Britton et al. [18] demonstrated significant positive independent associations of dietary magnesium intake with lung function, airway reactivity to inhaled methacholine and respiratory symptoms (wheezing) in the general population. Since most of the magnesium present in food is lost in cooking or refining, diets that provide a high proportion of daily energy requirements from refined or processed foods are likely to be low in magnesium [46]. Unfortunately, in Westernized countries processed food accounts for a substantial proportion of the diet, which increases the likelihood of true or relative magnesium deficiency [13]. This deficit in body magnesium may contribute to an increased contractility of smooth muscle in several organs and tissues like the vascular wall, inducing vasoconstriction, and producing bronchoconstriction in the bronchial smooth muscle cells [1,3,47].

It is noteworthy that some of the medications used in the treatment of asthma may affect the metabolism of magnesium, either directly or indirectly by affecting the metabolism of calcium, sodium or other ions. As such, it has been observed that theophylline given intravenously during asthma attacks increased total mean urinary excretion of magnesium, calcium and sodium [48]. However, in another study evaluating the effects of long-term oral β₂-agonists on magnesium and potassium levels in skeletal muscle biopsies, there was no significant difference between the measurements of the ions before and after 2 months of oral therapy. It is noteworthy that skeletal muscle magnesium in the patients with asthma was lower both before and after the therapy when compared with the non-asthmatic controls [49].

This is the first report relating intracellular magnesium concentrations and bronchial reactivity. Although a magnesium depletion during asthma attacks was first suggested [14], a recent report did not find any difference in intracellular magnesium concentrations between patients with asthma and normal controls [50]. In another study, Fantidis et al. [51] demonstrated a decreased magnesium content in polymorphonuclear cells from patients with asthma in resting conditions, between acute attacks. Although it is not easy to reconcile these contrasting results, a possible explanation for the differences observed is that most of these studies included subjects with dissimilar age ranges, which probably makes them incomparable, since intracellular magnesium varies substantially with age [52]. Nevertheless, our data clearly demonstrate that the intracellular content of magnesium is significantly and continuously connected to bronchial reactivity.

In conclusion, the strong relationship between intracellular magnesium levels and methacholine bronchial reactivity suggests that magnesium alterations may play a role in the pathogenesis of asthma bronchoconstriction and may help explain the favourable effects demonstrated after magnesium administration in patients with asthma.

ACKNOWLEDGMENT

This work was supported by a grant from Ministero dell’Università e della Ricerca Scientifica e Tecnologica (60%) to G.D.L.

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