Reduced Intracellular Mg Concentrations in Patients With Acute Asthma*

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Study objectives: To determine the intracellular and extracellular Mg concentrations in patients with acute asthma and their correlation with parameters expressing the disease severity.

Patients: Thirty patients with acute asthma (FEV₁, 56% predicted [SD, 14.5]), 20 patients with stable asthma (FEV₁, 97% predicted [SD, 10]), and 20 healthy subjects (FEV₁, 97% predicted [SD, 8]).

Methods: Mg concentrations in erythrocytes and plasma were measured four times: at hospital admission, after 2 days, after 5 days, and at hospital discharge. Percentage of predicted FEV₁ and peak expiratory flow rate variability were recorded simultaneously. Similar measurements were carried in all study groups.

Results: Mg concentrations of healthy subjects and patients with stable asthma remained unchanged in both plasma and erythrocytes. Initial Mg content in erythrocytes was significantly lower in patients with acute asthma (1.77 mmol per cell; 95% confidence interval [CI], 1.71 to 1.83) compared to normal subjects (1.94 mmol per cell; 95% CI, 1.82 to 2.00) and patients with stable asthma (1.92 mmol per cell; 95% CI, 1.87 to 1.96) [p < 0.0001], and it increased significantly after the resolution of the exacerbation (from 1.77 mmol per cell [95% CI, 1.71 to 1.83] at hospital admission to 1.90 mmol per cell [95% CI, 1.83 to 1.98] at hospital discharge; p < 0.0001). No correlation was observed between parameters of disease severity and the initial values of Mg concentrations in erythrocytes and plasma.

Conclusions: Acute asthma is associated with lower erythrocyte Mg content while plasma levels remain unchanged. This decrease in intracellular Mg content occurs regardless of the severity of the exacerbation and returns to normal values after control has been achieved.

(CHEST 2003; 123:113–118)

Key words: acute asthma; erythrocytes; magnesium

Abbreviations: AAS = atomic absorption spectrophotometry; CI = confidence interval; PEFR = peak expiratory flow rate

M

agnesium is the second most abundant intracellular cation, and it is a cofactor in > 3,000 enzymatic reactions involving energy metabolism and protein and nucleic acid synthesis.¹ Serum Mg contributes < 1% to the total amount of the body, and its function as a marker for Mg deficiency is doubtful. Therefore, an increasing interest can be noted in the measurement of its intracellular concentration.²³

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Manuscript received January 7, 2002; revision accepted July 16, 2002.

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Mg has been shown to relax bronchial smooth muscle in vitro⁴ and to bronchodilate asthmatic airways in vivo.⁵ On the basis of the critical role of Mg in the regulation of bronchial cell contractility via effects on calcium transport activation,⁶ and phosphorylation/dephosphorylation intracellular reactions,⁷ it has been proposed that the intracellular Mg content may determine the excitability of these cells.¹⁴ Mg deficiency may then lead to an increased excitability of bronchial smooth muscle with a consequent bronchoconstriction.⁵

Recent studies in acute and chronic asthma have shown no significant difference of plasma Mg concentrations in asthmatics and nonasthmatic control subjects.²⁹ Low intracellular Mg content in patients with stable asthma has been previously found,¹⁰–¹² but not all studies have confirmed this finding.²¹³ We have
previously shown that histamine challenge reduces Mg content in erythrocytes while plasma concentrations remain unchanged.10 This histamine-induced decrease in erythrocyte Mg content occurred regardless of the degree of bronchial reactivity and was attributed to histamine-induced bronchoconstriction. We hypothesized that a similar fall in intracellular Mg content might occur in patients with acute asthma, with exacerbation-induced bronchoconstriction. We therefore studied the concentrations of Mg in erythrocytes and plasma of patients with acute asthma and simultaneously investigated whether initial Mg concentrations were correlated with parameters expressing the disease severity. Similar measurements were performed in control groups (patients with stable asthma, normal subjects) in order to prove that the probable decrease of intracellular Mg might be mainly attributed to the exacerbation of the disease.

**MATERIALS AND METHODS**

**Patient Selection**

Subject characteristics are summarized in Table 1. Three different populations were studied. The first study population consisted of 30 patients with acute asthma (FEV1, 56% predicted [SD, 14.5]; range, 23 to 79% predicted). Two control groups of 20 patients with stable asthma (FEV1, 97% predicted [SD, 10]; range, 86 to 114% predicted) and 20 healthy subjects (FEV1, 97% predicted [SD, 8]; range, 87 to 112% predicted) were studied in order to assess the analytical and biological variation of the Mg assay. We used the recommendations of the National Heart, Lung, and Blood Institute workshop on the global strategy for asthma to classify patients as stable or exacerbated.14 Subjects with acute asthma received standard treatment with repetitive administration of short-acting nebulized β2-agonists and systemic glucocorticosteroids. None received Mg as a treatment. Patients with stable asthma were included in the study only if they were in clinically stable condition and had no evidence of acute exacerbation for at least 6 weeks prior to the study. Maintenance therapy of patients with stable asthma was the same with patients with acute asthma before the exacerbation of their disease, consisting of inhaled glucocorticosteroids (fluticasone, 250 to 500 μg/d; budesonide, 400 to 800 μg/d), with or without a long-acting inhaled β2-agonist. Healthy subjects were nonsmokers, with no history of atopy (negative skin-prick tests to common allergens) and were sex and age matched with asthmatic patients.

**Study Design**

Mg concentrations in plasma and in erythrocytes in the acute asthma group were measured at hospital admission before starting any treatment, at 2 days and 5 days after the initial measurement, and on the last day of hospital stay (mean day of discharge was day 7; range, 6 to 10 days). Percentage of predicted FEV1 and peak expiratory flow rate (PEFR) variability were recorded simultaneously as variables expressing the severity of the disease. Similar measurements were performed in the control groups using the same time intervals. The last sample in control groups was drawn on day 7, the mean day of discharge of patients with acute asthma.

All of the subjects had the same diet for at least 8 weeks before entering the study because they were officers and soldiers of the Greek army, and their meals were the common dietary program of the Greek army. The protocol was approved by the hospital ethics committee, and informed consent was obtained from each subject.

**Lung Function Tests**

FEV1 was measured using a dry bellows spirometer (44000 series; Vitalograph; Buckingham, UK). At least three reproducible values were obtained, with the highest value used for analysis. Diurnal PEFR variation was calculated by the following formula: (evening PEFR – morning PEFR)/mean of evening and morning PEFR) times 100. The results are shown as mean daily variation.

**Mg Measurements**

Venous blood samples were obtained without tourniquet from all subjects in sitting position. The aspirated blood was trans-
ferred to a metal-free test tube containing sodium heparin, and a CBC count was measured immediately. Plasma was separated from the erythrocytes within 30 min (650 g for 10 min). Of the isolated erythrocytes, 100 μL was aspirated and lysed with 1.5 mL of double-distilled water. Samples of plasma with hemo-

ysis or lipemia were excluded from the study. The Mg concentrations in plasma and in RBCs were determined by a colorimetric assay using calmagite, a metalchromic dye. In plasma, the measurements were carried out without deproteinization, and in RBCs after deproteinization using sodium tungstate at an acid pH (the lysed erythrocytes were reacted with 200 μL 0.3 mol/L Na2WO4 and 200 μL 0.35 mol/L H2SO4). Briefly, 1 mL of working solution (0.08 g/L calmagite, 0.04 g/L ethylene glycol-

bin-N,N',N'-tetra acetic acid, 0.28 mM KCl, 0.34 mM/L KCl, 0.98 g/L Bion Ne-9, and 0.001 g/L Bion PVP; Bion; Lancer Division of Sherwood Medical; St. Louis, MO) reacted with 20 μL of the plasma or 200 μL of the supernatant from the erythrocyte pellet deproteinization for 2 min at room tempera-

ture. The absorbance of the reaction product, a pink magnesium-

calamgite complex, was measured at 532 nm, using a double-

beam spectrophotometer (model UVIKON 940; Kontron Instruments; Zurich, Switzerland), against a “blank solution.” Measurements were quantitated using standard solutions. Blank solution consisted of a 1-mL working solution and 20 μL of double-distilled water, for plasma measurements; and 1-mL working solution, 160 μL of double-distilled water, 20 μL 0.3 mol/L Na2WO4, and 20 μL 0.35 mol/L H2SO4, for RBC measurements. The interference by possible magnesium traces in the reagents (Na2WO4, H2SO4) was avoided by using the aboved

mentioned reagent blank with every analytic procedure. The investigator who performed the magnesium measurements was not aware of the clinical and functional status of the subjects.

Statistical Analysis

Three populations were studied: patients with acute asthma, patients with stable asthma, and healthy subjects. A Kolmogorov-

Smirnov test was applied for skewedness of distribution, and all data gave a Gaussian distribution of deviation. Differences of Mg concentrations between the four measurements in each study population were tested with one-way analysis of variance followed by appropriated post hoc test (Bonferroni) for multiple comparisons in the case of significant overall effect. Differences of Mg concentrations between study populations were tested with one-way analysis of variance fol-

lowed by appropriated post hoc test (Bonferroni) for multiple comparisons. Correlation coefficient (r) [Pearson test] was ap-

plied to test the correlation between plasma and erythrocyte Mg and percentage of predicted FEV1 and PEFR variability, respec-

tively.

Data concerning the subjects characteristics are shown as mean (SD) and range. Data concerning the comparisons among the various parameters in the study groups are given as mean with 95% confidence intervals (CIs) for the differences. A p value < 0.05 was considered significant. All analyses were performed with SPSS (version 8.0; SPSS; Chicago, IL).

Results

Initial Mg concentrations in plasma did not significa-

antly differ between the three study populations: mean, 1.97 mg/dL (95% CI, 1.91 to 2.03) in patients with acute asthma; mean, 1.97 mg/dL (95% CI, 1.87 to 2.07) in patients with stable asthma; and mean, 2.01 mg/dL (95% CI, 1.90 to 2.13) in healthy subjects (p = 0.79; Fig 1). However, initial Mg content in erythrocytes was significantly lower in patients with acute asthma (1.77 fmmol per cell [95% CI, 1.71 to 1.83]) compared to control groups: mean, 1.92 fmmol per cell (95% CI, 1.87 to 1.96) in patients with stable asthma; mean, 1.94 fmmol/cell (95% CI, 1.82 to 2.00) in healthy subjects (p < 0.001; Fig 1). Initial erythrocyte Mg content in patients with acute asthma (mean, 1.77 fmmol per cell [95% CI, 1.71 to 1.83]) was significantly increased compared to the baseline value on day 2 (mean, 1.83 fmmol per cell [95% CI, 1.77 to 1.89]), day 5 (mean, 1.89 fmmol per cell [95% CI, 1.82 to 1.96]), and on hospital discharge (mean, 1.91 fmmol per cell [95% CI, 1.83 to 1.98]) [p < 0.000; Fig 2]. As a result of this increase, no significant difference was found in the Mg content in erythrocytes on the last sample between the three study populations: mean, 1.91 fmmol per cell (95% CI, 1.83 to 1.98) in patients with acute asthma; mean, 1.94 fmmol per cell (95% CI, 1.90 to 1.97) in patients with stable asthma; and mean, 1.94 fmmol per cell (95% CI, 1.83 to 2.05) in healthy subjects (p = 0.78). Mg content in erythrocytes in both control groups remains unchanged within the time intervals of all measurements (Fig 2). Initial RBC volumes were similar among the three groups: mean, 5.227 mol/L/μL (95% CI, 5.037 to 5.418) in patients with acute asthma; mean, 5.267 mol/L/μL (95% CI, 4.876 to 5.658) in patients with stable asthma; and mean, 5.266 mol/L/μL (95% CI, 4.893 to 5.639) in healthy subjects (p = 0.96; Table 1), and did not have a statistically significant difference within the time intervals of all measurements. Mg concentrations in plasma and in erythrocytes did not significa-

tly correlate with percentage of predicted FEV1.
cytes) in patients with acute asthma.

Steroids and inhaled controller asthmatic therapy (inhaled glucocorticoids) in Mg status of the patients. No significant correlation was observed in the majority of them, no difference was found in erythrocyte Mg content between patients with stable asthma and healthy subjects. The only study to our knowledge dealing with Mg status in asthmatic patients found in erythrocyte Mg content between patients with stable asthma and healthy subjects.

Although often correlated, serum concentrations and intracellular content of Mg can be dissociated and signs of Mg deficiency occur with normal or minimally low serum concentrations. Therefore, many studies have shown the superiority of measuring the intracellular content of Mg over measuring serum concentrations in determining the Mg status of patients. Intracellular content were measured in erythrocytes, as these cells represent the largest subcellular compartment of blood cells, they contain Mg in ionized-diffusible form, and they have been widely used in clinical studies. Furthermore, it has been shown that erythrocytes provide an experimental model that allows repeatable and accurate measurements of intracellular ions and reflects Mg stores in patients with bronchial asthma better than other circulating blood cells. We measured total Mg in both plasma and erythrocytes, despite the fact that ionized Mg is the active form of the ion. We believe that measurements of ionized Mg have still some analytical and methodologic problems, not widely used in clinical practice, and measuring ionized Mg would lead to incompatible results in regard to those previously reported in the literature for asthmatic patients.

Atomic absorption spectrophotometry (AAS) is the preferable method for the determination of Mg in biological specimens, but it is not a usual method in clinical laboratories. According to the College of American Pathologists Comprehensive Chemistry Survey for 1991, calmagite was used by 43% of laboratories for Mg determination while AAS was used by only 1%. So the calmagite colorimetric method (which was used in this study) is fairly accurate and precise and is widely used in clinical laboratories, with a good correlation compared to AAS.

Many studies have been involved with the Mg status in asthmatic patients and the role of IV or nebulized MgSO4 in acute asthma, with conflicting results. In the majority of them, no difference was found in erythrocyte Mg content between patients with stable asthma and healthy subjects. The only study to our knowledge dealing with Mg status in acute asthma reported that serum concentrations in asthma patients with exacerbations were similar to the control nonasthmatic subjects. Our study confirmed these results and additionally showed the presence of reduced intracellular Mg content in patients with acute asthma. We believe that these reduced levels are mainly attributed to the exacerbation-induced bronchoconstriction. This is supported by the absence of biological or analytical variation of Mg concentration in the control groups, and the significant increase of erythrocyte Mg concentration in patients with acute asthma after the resolution of the exacerbation. This recovery of

Discussion

This study has shown that acute asthma is associated with lower erythrocyte Mg content while plasma levels remain unchanged. This fall in intracellular Mg content was mainly attributed to the exacerbation-induced bronchoconstriction, since Mg concentration did not significantly change within all the measurements in control groups. In addition, the latest data have shown no effect of the regular daily concentration did not significantly change within all the measurements in control groups. In addition, the latest data have shown no effect of the regular daily exacerbation-induced bronchoconstriction, since Mg concentration did not significantly change within all the measurements in control groups. In addition, the latest data have shown no effect of the regular daily exacerbation-induced bronchoconstriction, since Mg concentration did not significantly change within all the measurements in control groups. 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intracellular Mg can be mainly attributed to the reverse of the bronchoconstriction since therapeutic agents that are used to treat patients with acute asthma (corticosteroids and short-acting β₂-agonists) seem to have the opposite (negative) effect on the Mg status of the patients.29,30 Additionally, these results are in agreement with our previous study,13 which showed that during bronchial challenge with histamine, where an “experimental” bronchoconstriction occurs, intracellular Mg content showed a significant fall. Therefore, we believe that the presence of either experimental or exacerbation-induced bronchoconstriction leads to the same result, which is a significant fall in erythrocyte Mg content. This significant fall seems to be independent of both the degree of bronchial hyperresponsiveness and the severity of the exacerbation.

All the above-mentioned data could possibly lead to the hypothesis that the reduced Mg levels in erythrocytes—and in intracellular space in general—is the result and not the cause of bronchoconstriction (produced with provocative agents or environmental stimuli) in an asthmatic exacerbation. Our speculation is that when bronchoconstriction occurs, Mg is forced out of intracellular space and used as a natural calcium-channel blocker in order to relax airway smooth muscle. One plausible explanation for the above-mentioned theory might be that products of oxidative stress produced in asthma exacerbation, like hydrogen peroxide (H₂O₂),31 can possibly act as indirect triggers—destroying Na/Mg adenosine triphosphatase antiport32—to pour Mg out of erythrocytes. Other inflammatory chemokines, such as interleukins, might take part to this process, through cyclic adenosine monophosphate regulation.33 This is partially supported by the results of in vivo34,35 and in vitro7 studies that showed that IV MgSO₄ produced a rapid and marked but temporary smooth-muscle relaxation, which lasts only during Mg infusion period, without changing intracellular Mg content. Additionally, other studies showed that increased dietary Mg intake failed to demonstrate improvements in lung function or airway reactivity.36

**CONCLUSION**

In summary, acute asthma is associated with lower erythrocytes Mg content, while plasma Mg concentrations remain unchanged. These reduced intracellular contents are not correlated with the severity of asthma exacerbation and they return to normal values after the treatment of the exacerbation. Further studies are needed in order to understand better the altered Mg homeostasis in asthmatic patients and the role of Mg membrane antiport system.

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