Inhaled magnesium fluoride reverse bronchospasma

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Abstract

Asthma is a global health problem. Asthma attacks are becoming more severe and more resistant to usual treatment by β_2 agonists nebulisation. The search for a new product that could reduce the morbidity of asthmatic disease seems necessary. The objective of this study was to compare the effectiveness of inhaled magnesium fluoride (MgF_2) with that of magnesium sulphate (MgSO_4) 15% alone and sodium fluoride (NaF) 0.5 M alone in rats pre-contracted by methacholine (MeCh). Fifty six adult male Wistar rats of medium weight 259 ± 15 g were divided randomly into five groups. They inhaled respectively: MeCh, MgF_2 + NaCl 0.9%, MgF_2 + acetic acid, MgSO_4 15% single and NaF (0.5 M) single. Airway resistances were measured after each dose of MeCh by pneumomultitest equipment. Results indicated that (1) MgF_2 + NaCl 0.9%, MgF_2 + acetic acid and MgSO_4 reversed significantly the methacholine-induced bronchial constriction in rats and had a bronchodilating effect at the moment of its administration (2) MgF_2 + acetic acid led to a greater decrease ($P < 0.05$) of bronchial resistances when compared to that obtained from MgF_2 + NaCl 0.9%, NaF exclusively and MgSO_4 alone (3) inhaled NaF alone led to a significant bronchorelaxing effect ($P < 0.05$) that starts at the sixth dose of MeCh (17 mg/L). As a matter of fact, MgF_2 dissolved in acetic acid and delivered in aerosol form reduces significantly bronchial spasm. In conclusion, MgF_2 can be used as a bronchodilator for diseases with bronchospasma such as asthma and chronic obstructive pulmonary disease (COPD).

Key words: magnesium fluoride, sodium fluoride, sulphate magnesium, airway resistance, rat

Introduction

Despite the progress made on the management of asthma, the prevalence, morbidity and mortality of this disease seems constantly increasing. During an asthma attack, the smooth muscle in the airway contracts more than it should, which causes an increase of bronchial resistances values (R) and so dyspnea (Stephens, 2002).

NaF is an inhibitor of enolase, an enzyme of the glycolysis pathway leading to phosphoenolpyruvate. Oral NaF was initially examined in the treatment of osteoporosis by

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Charles *et al.* (1989). Cushing *et al.* (1990) found that NaF relaxed arteries by releasing an endothelium derived relaxing factor and one or more prostanoids. Whereas, inhaled effect of NaF was poorly documented. It had been shown that inhaled NaF, induce bronchial relaxation on pre contracted bovine bronchi *in vitro* and in rats *in vivo* (Zhao and Guénard, 1997; Zhao *et al.*, 2002).

MgSO₄ is an agent that has been given as an additive treatment of patients with acute asthma and has been shown to be effective in patients with severe acute asthma when delivered parentally (Rowe *et al.*, 2000). Magnesium may be effective in acute asthma through one or more of a variety of mechanisms. Magnesium relaxes smooth muscle and inhibits the smooth muscle contraction (Gourgoulisanis *et al.*, 2001). This theory has been proposed as an explanation for the beneficial effects of MgSO₄ in patients with acute asthma. However, this explanation may be too simplistic. Magnesium is also involved with cellular homeostasis through its role as an enzymatic cofactor, as well as being involved in acetylcholine and histamine release, from cholinergic nerve terminals and mast cells, respectively. Investigators have proposed that the effect of MgSO₄ is related to its ability to block the calcium ion influx to smooth muscles of the respiratory system (Gourgoulisanis *et al.*, 2001). Finally, the role of MgSO₄ as an anti-inflammatory has been identified in adults with asthma (Carins and Kraft, 2001).

The use of MgSO₄ is one of numerous options available during exacerbations of asthma (Noppen, 1990). While the efficiency of intravenous MgSO₄ in these cases has been demonstrated, little is known about inhaled MgSO₄. The potential clinical benefit of inhaled MgSO₄ has been studied, and research publications have produced conflicting results (Hill *et al.*, 1997; Rolla *et al.*, 1988). Consequently, this agent is not currently recommended as part of the current guidelines and has not been used widely in most acute cases. The few times that inhaled magnesium has been mentioned, it has been as a minor effect (Harari *et al.*, 1998).

Fluoride and magnesium ions (F⁻ and Mg²⁺) associated in MgF₂ could have a major bronchorelaxing effect. In theory, these two ions act additively and synergically, accordingly MgF₂ could be an efficient pharmacological compound against bronchospasm. The bronchorelaxing effect of this product MgF₂ has never been tested.

In Tunisia, where this research was conducted, MgF₂ is an abundant mineral and inexpensive element that could be useful for the treatment of asthmatics.

The purpose of this study was to compare bronchodilating effects obtained by inhaled compounds: NaF, MgSO₄ and MgF₂.

Subjects and Methods

Subjects

Fifty six adult male Wistar rats (weight, 259 ± 15 g; mean ± SE). Rats were divided randomly into five groups treated with the following drugs respectively: methacholine (MeCh) (n=12), MgF₂ + NaCl 0.9% (n=13), MgF₂ + acetic acid (n=12), NaF 0.5 M (n=8) and MgSO₄ (n=11).

Rats were anesthetized intraperitoneally with ketamine (150 mg/kg). After dissecting the neck, a tracheal cannula was inserted into a mid-line incision of the trachea. A catheter was inserted into oesophagus and connected to a pressure transducer to measure the intra-oesophageal pressure. A small pneumotachograph (PTG, 8431B, Hans Rudolph, Kausas, USA) was connected

to tracheal cannula. The period of measurement of the flow rate with the PTG was set at 10 sec to avoid change in ventilation due to the PTG dead volume. The PTG was connected to a differential pressure transducer. Both pressure and flow transducers were assembled together with connecting valves to ease the calibration. Calibration in volume was done daily with a 10 ml syringe. Total lung resistance (R) was calculated by using a first order mechanical model of the lung. Aerosolizations were made through a DeVilbiss nebulizer (Ref. 123016 Marquette Medical products, Englewood Co., USA) connected to a compressor (flow rate 100 ml/s). Aerosols were delivered at a flow rate of 0.1 ml/s in a rigid plastic chamber placed over the rat body (Zhao *et al.*, 2002).

Bronchoconstriction was induced by gradually increasing concentrations of MeCh: 0.5 mg/L, 1 mg/L, 2.12 mg/L, 4.25 mg/L, 8.5 mg/L, 17 mg/L, 34 mg/L and 68 mg/L. MeCh solutions were aerosolized within the chamber for 1 min with 3 min intervals between doses.

(MgF₂ + NaCl 0.9%), (MgF₂ + acetic acid), NaF and MgSO₄ inhaled aerosols were delivered for one minute after each dose of MeCh from the fourth dose of MeCh. The total lung resistances (R) were measured before the challenge, after an aerosol of isotonic saline and 2 minutes after each dose of MeCh.

Protocols

Animal experiment protocols used in the present study were approved by the Animal Ethics Committee of the Faculty of Medicine of Sousse, Tunisia, where the experiments were carried out. Five protocols were made:

Protocol 1: MeCh was administrated to (MeCh) group, (n=12) at increasing doses (0.5, 2.12, 4.25, 8.5, 17, 34, 68 mg/L). The total lung resistance (R) was measured after each dose of MeCh.

Protocol 2: The rats of the (MgF₂ + NaCl 0.9%) group, (n=13) were firstly challenged by increasing doses of MeCh. MgF₂ dissolved in NaCl 0.9 % (Mg=0.04 mM, F=0.014 mM) in aerosol form was delivered at the fourth dose of MeCh (4.25 mg/L).

Protocol 3: The rats of the (MgF₂ dissolved in acetic acid) group, (n=12) were firstly challenged by increasing doses of MeCh. Then, MgF₂ dissolved in acetic acid (Mg=0.08 mM, F=0.084 mM) was delivered in aerosol form at the fourth dose of MeCh (4.25 mg/L).

Protocol 4: The (NaF) group, (n=8) was firstly challenged by increasing doses of MeCh. NaF (0.5 M) exclusively, in aerosol form, was delivered at the fourth dose of MeCh (4.25 mg/L).

Protocol 5: The (MgSO₄) group, (n=11) was firstly challenged by increasing doses of MeCh, MgSO₄ 15% single, in aerosol form, was delivered at the fourth dose of MeCh (4.25 mg/L).

Chemicals used

MgSO₄, NaF, acetic acid and ketamine were purchased from Sigma (St. Louis, MI, USA) and methacholine from Allerbio (La varenne, France). NaF was dissolved in distilled water devoid of traces of aluminium. MgF₂ was dissolved in acetic acid to improve the solubility. Magnesium fluoride, random crystals, 99.99+%, optical grade: purchased from (Sigma, Aldrich).

Data analysis

All data were reported as a mean $\pm$ SEM. A *P* value <0.05 was considered significant. Mean

values of R between control and other groups were compared using the Mann-Whitney U test. Comparison of rat's resistances (R) values among the same group of rats at different concentrations of MeCh was made using the paired Student's *t*-test. Changes in R during the methacholine challenge in different groups were analysed with a two-way ANOVA.

Results

Effects of methacholine inhalation on (R) values

After the inhalation of methacholine, (R) values increased proportionally with increases in the methacholine concentration. Figure 1 shows the (R) values at different concentrations of methacholine. A significant increase in bronchial resistance ($P < 0.05$) at the fourth dose of methacholine (4.25 mg/L) was observed (Fig. 1).

Effects of MgF_2 inhalation on (R) values

Inhaled (MgF_2 + NaCl 0.9%) and (MgF_2 + acetic acid) reversed significantly the methacholine-induced bronchial constriction in rats at the moment of its administration (fourth dose of MeCh, 4.25 mg/L) (Fig. 1).

The dissolution of MgF_2 in acetic acid led to an increase in the total molar concentrations of magnesium and fluoride in the solution compared to its dissolution in NaCl 0.9%: MgF_2 dissolved in acetic acid ($Mg = 0.08$ mM, $F = 0.084$ mM, solubility $= 0.126 \cdot 10^{-3}$) MgF_2 + NaCl 0.9% ($Mg = 0.04$ mM, $F = 0.014$ mM, solubility $= 0.521 \cdot 10^{-3}$). MgF_2 pure crystals 99.99+% were used to avoid any contaminations with other elements which could fault the results like calcium that plays an important role in the contraction of smooth muscle.

Inhaled (MgF_2 + acetic acid) led to a greater decrease ($P < 0.05$) of (R) values, when compared to those obtained with MgF_2 + NaCl 0.9% (Fig. 1).

The comparison between (MgF_2 + NaCl 0.9%) and (MgF_2 + acetic acid) groups using Mann-Whitney U-test revealed significant difference ($P < 0.05$) in the three highest concentrations of MeCh (17, 34, 68 mg/L).

Effects of NaF (0.5M) inhalation on (R) values

Inhaled NaF, administrated at the fourth dose of methacholine, led to a meaningful bronchorelaxing effect ($P < 0.05$) that starts at the sixth dose of MeCh (17 mg/L) (Fig. 2).

Effects of $MgSO_4$ (15%) inhalation on (R) values

Inhaled $MgSO_4$ 15% reversed significantly ($P < 0.05$) the methacholine-induced bronchial constriction in rats at the moment of its administration (fourth dose of MeCh, 4.25 mg/L) (Fig. 2).

Discussion

The main findings of this study were: (1) MgF_2 + NaCl 0.9%, MgF_2 + acetic acid and $MgSO_4$ reversed significantly the methacholine-induced bronchial constriction in rats and had a bronchodilating effect at the moment of its administration (2) MgF_2 + acetic acid led to a greater

Fig. 1. Effects of inhaled MgF₂ + NaCl 0.9% (n=13) and MgF₂ + acetic acid (n=12), on airway resistances (R) of rat challenged by methacholine (MeCh) (n=12). *, $P < 0.05$, significant increase in bronchial resistance that starts at the fourth dose of MeCh (4.25 mg/L). **, $P < 0.05$, Mann-Whitney U test. The comparison between (MgF₂ + NaCl 0.9%) and (MgF₂ + acetic acid) groups using Mann-Whitney U test revealed significant differences in bronchial resistance ($P < 0.05$) that starts at the sixth dose of MeCh (17 mg/L).

Fig. 2. Effects of inhaled MgSO₄ (n=11), NaF 0.5M (n=8) and MgF₂ + acetic acid (n=12), on airway resistances (R) of rat challenged by methacholine (MeCh) (n=12). *, $P < 0.05$, Mann-Whitney U test. The comparison between (MgF₂ + acetic acid) and NaF groups using Mann-Whitney U test revealed significant differences in bronchial resistance ($P < 0.05$) that starts at the fourth dose of MeCh (4.25 mg/L). **, $P < 0.05$, Mann-Whitney U test. The comparison between (MgF₂ + acetic acid) and MgSO₄ groups using Mann-Whitney U test revealed significant differences in bronchial resistance ($P < 0.05$) that starts at the fourth dose of MeCh (4.25 mg/L).

decrease ($P < 0.05$) of bronchial resistance when compared to those obtained with MgF₂ + NaCl 0.9%, NaF alone and MgSO₄ alone (3) inhaled NaF alone led to a meaningful bronchorelaxing effect ($P < 0.05$) that starts at the sixth dose of MeCh (17 mg/L).

The anaesthesia of rat by Ketamin was based on its easy administration, low toxicity, good conservation of ventilation and its very high lethal doses on animals (Riou and Ducart, 1994; Dureuil, 1996). Methacholine was used to challenge the rat before any administration. In fact, methacholine was reported as a synthetic muscarinic agonist more stable than acetylcholine

(destroyed by the cholinesterase) and better tolerated than histamine or carbachol. In addition, MeCh rarely induced a cough (Wanger, 1996; Sterck *et al.*, 2001).

NaF is less potent than aluminium fluoride (AlF_4^-) in the activation of G proteins. AlF_4^- mimics the action of GTP at micromolar concentrations by inducing dissociation of the α subunit of G protein followed by the calcium channel modulation (Stadel and Crooke, 1988). A possible contamination of Al from the glass ware might have affected the results obtained with NaF. When F^- is diluted in the solution, some Al is extracted from the surface of the glass to form AlF_4^- . This phenomenon was excluded in the present study because all fluoride solutions were prepared and stored in polyethylene bottles in order to prevent attack on glass surfaces. The choice of NaF dose (0.5 M) was based on findings of Zhao *et al.*, 2002 which demonstrated that at this concentration we obtained a decrease of airway resistances (Zhao *et al.*, 2002). All compounds: ($\text{MgF}_2 + \text{NaCl}$ 0.9%), ($\text{MgF}_2 + \text{acetic acid}$), NaF and MgSO_4 were administered in inhaled form because this mode of administration did not require careful monitoring. In fact, in intravenous administration peripheral vasodilatation and systolic hypotension can occur and patients sometimes have unpleasant flushing, nausea, and venous phlebitis from the infusion.

The results of this study were in agreement with previous studies of Zhao *et al.* and Rolla and colleagues (Rolla *et al.*, 1987; Rolla *et al.*, 1988) that confirmed the bronchorelaxant effect of inhaled NaF 0.5 M and inhaled MgSO_4 respectively. The use of NaF as a therapeutic agent for asthma disease is very limited despite its bronchodilator effect demonstrated at well-defined dose both *in vitro* and *in vivo* by Zhao and Guénard (1997) and Zhao *et al.* (2002). This encourages us to explore the inhaled effect of NaF and try to determine the causes of these differences in the results. The bronchodilator effect of NaF is thus far poorly documented. NaF had been reported by (Stadel and Crooke, 1988; Cushing *et al.*, 1990) to stimulate adenylate cyclase activity on smooth muscles and induced NO synthesis which would relax bronchi. The better known bronchodilator mechanism of NaF is induced by inhibition of the glycolytic enzyme, enolase, which converts 2-phosphoglycerate to phosphoenolpyruvate according to (Zhao and Guénard, 1997). The inhibition of glycolysis induced by NaF is illustrated by the sharp decrease in lactate production in its presence (Zhao *et al.*, 2002). Inhibition of this enzyme would be expected to reduce glycolytic ATP production and impair smooth muscle contraction.

While the effect of MgSO_4 administered intravenously has been confirmed by several studies (Noppen *et al.*, 1990; Rowe *et al.*, 2000; Gourgouliaanis *et al.*, 2001), its effect through inhalation is controversial. In fact, while some studies confirm its beneficial effect (Rolla *et al.*, 1987; Rolla *et al.*, 1988), others deny it (Hill *et al.*, 1997). This controversy of results could be related to the serum magnesium levels of patients. Alamoudi' study (Almuodi, 2000) confirmed that hypomagnesaemia is common in chronic asthmatics. Chronic asthmatics with low Mg tend to have more hospitalizations than chronic asthmatic with normal Mg. Hypomagnesaemia was also associated with more severe asthma. Then, routine serum magnesium determination is recommended in patients with chronic obstructive lung disease. The lack of inhaled MgSO_4 studies and its effects and the controversial results obtained leaves us both surprised and wary of such treatment. However, it does not deny in any case the bronchorelaxant effect of magnesium.

MgSO_4 has been reported in many researches to inhibit the Ca^{2+} influx by blocking the voltage-dependent calcium channels, modulate vasoactivity by affecting the influx of extracellular

Ca²⁺ through dihydropyridine-sensitive, voltage-dependent channels, which accounts for much of its relaxant action on airway (Sharma *et al.*, 1994; Hirota *et al.*, 1999). *In vitro* studies showed that magnesium ion (Mg²⁺) modulates smooth muscle contractility and mediates release by antagonism of the action of calcium (Sonna *et al.*, 1996).

Owing to this, the association of the two trace elements magnesium (Mg) and fluoride (F) in the form of MgF₂ could represent a potential new therapeutic treatment for asthma. MgF₂ was rarely used because of the low solubility of this compound which was estimated to 10⁻⁵ (Fovet and Gal, 2000). In this study, we associated MgF₂ to acetic acid that increased its solubility. In fact, the dissolution of MgF₂ in acetic acid led to an increase of the total molar concentrations of magnesium and fluoride in the solution compared to its dissolution in NaCl 0.9%: MgF₂ dissolved in acetic acid (Mg=0.08 mM, F=0.084 mM, solubility=0.126.10⁻³) MgF₂ + NaCl 0.9% (Mg=0.04 mM, F=0.014 mM, solubility=0.521.10⁻³). MgF₂ pure crystals 99.99+% were used to avoid any contaminations with other elements which could fault the results like calcium that plays an important role in the contraction of smooth muscle.

MgF₂ dissolved in acetic acid reversed significantly methacholine bronchial responsiveness in rats challenged by MeCh. This result confirmed the bronchorelaxant effect of MgF₂. Moreover, MgF₂ had the best bronchorelaxant effect compared to NaF (0.5 M) alone or MgSO₄ alone. Then, the association of magnesium and fluoride makes a powerful bronchodilator that acts at a micromolar dose range compared to NaF 0.5 M or MgSO₄ (15%) alone.

In the present study, inhaled doses of MgSO₄, NaF and MgF₂ were very low and far from the toxic doses of magnesium and fluoride (Spencer *et al.*, 1981; Whitford, 1996; Akiniwa, 1997). It is difficult to assess the dose of MgF₂ needed for the treatment of asthma as this study seems to be the first to demonstrate its bronchodilating effect *in vivo*. The duration of the bronchorelaxant effect of MgSO₄, NaF and MgF₂ is not known and needs further study.

Then, we conclude that magnesium and fluoride in salt form: MgSO₄, NaF and MgF₂ pure crystals act as bronchodilator. MgF₂ pure acts and has a bronchodilating effect by releasing a micromolar dose range of magnesium and fluoride (Mg=0.08 mM, F=0.084 mM). Indeed, owing to its two different but complementary and synergic bronchorelaxant ways to induce bronchorelaxing effect. Inhaled MgF₂ can reverse bronchospasms in case of asthma. In Tunisia, where this research was conducted, MgF₂ is an abundant mineral and could be a useful and inexpensive tool for the treatment of asthmatics. Which, therapeutics cost is increasing constantly.

In conclusion, the present study showed that inhaled MgF₂ relaxed methacholine bronchoconstriction in rats. Yet, in the light of these experiments on rats and the positive results achieved, we can move to clinical essays of MgF₂ by testing its effects on bronchial hyper responsiveness.

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