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ORIGINAL ARTICLE

Functional and histological effects of inhaled magnesium alone or associated to fluoride: An experimental study in rats

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Abstract The study was carried out to investigate the effects of inhaled Mg alone and associated with F in the treatment of bronchial hyperresponsiveness. 43 male Wistar rats were randomly divided into four groups and exposed to inhaled NaCl 0.9%, MeCh, $MgSO_4$ and MgF_2. Pulmonary changes were assessed by means of functional tests and quantitative histological examination of lungs and trachea. Results revealed that delivery of inhaled Mg associated with F led to a significant decrease of total lung resistance better than inhaled Mg alone ($p < 0.05$). Histological examinations illustrated that inhaled Mg associated with F markedly suppressed muscular hypertrophy ($p = 0.034$) and bronchoconstriction ($p = 0.006$) in MeCh treated rats better than inhaled Mg alone. No histological changes were found in the trachea. This study showed that inhaled Mg associated with F attenuated the main principle of the central components of changes in MeCh provoked experimental asthma better than inhaled Mg alone, potentially providing a new therapeutic approach against asthma.

Introduction

Asthma is a serious global health problem, throughout the world affecting over 300 million people of all ages. Asthma is a chronic disease characterized by a variety of features, including increased airway responsiveness, airway inflammation and reversible airway obstruction. According to the Global Initiative for Asthma (GINA), the definition of asthma is based on an operational description. In these terms:

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"*Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment*" [1].

Several studies [2–4], had confirmed the bronchodilating effects of intravenous Mg, but its effects through inhalation are controversial [5–8]. Mg has been reported in many researches to inhibit the Ca^{2+} influx by blocking the voltage-dependent calcium channels, modulate the vaso-activity by affecting the influx of extracellular Ca^{2+} through dihydropyridine-sensitive, voltage-dependent channels, which accounts for much of its relaxing action on airway [9,10]. In vitro studies showed that the magnesium ion (Mg^{2+}) modulates smooth muscle contractility and mediates release by antagonism of the action of calcium [11,12].

The bronchodilator effect of fluoride is poorly documented. Cushing et al., found that NaF relaxed arteries by releasing an endothelium derived relaxing factor and one or more prostanoids [13]. Zaho et al. proved that NaF induced bronchial relaxation on precontracted bovine bronchi in vitro and rats in vivo. In fact, fluoride is an inhibitor of enolase, an enzyme of the glycolysis pathway leading to phosphoenolpyruvate [14,15].

As compared with physiological studies, there are a few detailed histological studies on the respiratory system, especially in the lung, of humans or rats exposed by inhalation to Mg alone or when associated with fluoride.

The purposes of this study were to investigate the changes in the pulmonary function as well as in the histology in the lung of Wistar rats which were challenged with MeCh and then exposed by inhalation to Mg alone and when associated with fluoride.

Materials and methods

The study protocol used in the present study was approved by the Animal Ethics Committee of the Faculty of Medicine of Sousse, Tunisia, where the experiments were carried out.

Animals

Forty-three male Wistar adult rats (180 g ± 30 g) were included in the study. Rats were randomly assigned to four groups: Controls ($N = 14$), MeCh alone ($N = 10$), $MgSO_4$ ($N = 9$) and MgF_2 ($N = 10$).

Total lung resistances measurement

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 the esophagus and connected to a pressure transducer to measure the intra-esophageal pressure. A small pneumotachograph (PTG, 8431B, Hans Rudolph, Kansas, USA) was connected to tracheal cannula. The period of measurement of the flow rate with the PTG was set at 10 s to avoid a 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. Aerosols 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. 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.

$MgSO_4$ and MgF_2 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 min after each dose of MeCh.

Histology

At the end of the protocols, the rats were sacrificed; the lungs and trachea were removed. Longitudinal sections were taken from the left and right lung and trachea sections were cut transversely. The lungs and trachea sections were processed by routine histological procedures for paraffin embedding. Five-micrometer thick histological sections were stained with hematoxylin and eosin, and examined by light microscopy. Histological modifications of the lungs and trachea were assessed by means of a quantitative histological score. The degrees of inflammation, mucus, muscular hypertrophy, bronchial dilatation and emphysema were scored from 0 (absent) to 3 (intense) by two pathologists who examined the slides at the same time under a double-observation microscope. The histological slides were coded and the two investigators were unaware of the origin of the material during scoring.

Chemicals

$MgSO_4$, acetic acid and ketamine were purchased from Sigma (St. Louis, MI, USA) and MeCh from Allerbio (Lavarenne, France). MgF_2 was dissolved in acetic acid to improve the solubility.

Magnesium fluoride, random crystals, 99.99 + %, optical grade: purchased from (Sigma, Aldrich).

Solutions synthesis

MgF_2 was dissolved in acetic acid to improve the solubility. MgF_2 solution was prepared and stored in polyethylene or polypropylene bottles in order to prevent attack on glass surfaces.

Data analysis

All data are reported as mean ± SEM. Mean values of R between control and other groups were compared using the Mann–Whitney's U test. Comparison of rat's resistance (R) values among the same group of rats at different concentrations of MeCh was made using the paired Student's t-test.