European Journal of Pharmacology 801 (2017) 79–85

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Effects of nebulised magnesium sulphate on inflammation and function of the guinea-pig airway

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ARTICLE INFO

Keywords:
Magnesium sulphate
Asthma
Bronchoprotection
Guinea-pigs
Airways inflammation
Lipopolysaccharide

ABSTRACT

Magnesium sulphate is a potential treatment for acute severe asthma. However, the mechanisms and dose-response relationships are poorly understood. The first objective of this study was to examine whether inhaled magnesium sulphate exerts bronchodilator activity measured as bronchoprotection against histamine-induced bronchoconstriction in conscious guinea-pigs alone and combined with salbutamol. Secondly, we examined whether inhaled magnesium sulphate inhibits airways inflammation and function in models of neutrophilic and eosinophilic lung inflammation induced, respectively, by inhaled lipopolysaccharide or the inhaled antigen, ovalbumin (OVA). Airway function was measured in conscious guinea-pigs as specific airway conductance (sG_{aw}) by whole-body plethysmography. Anti-inflammatory activity was measured against lung inflammatory cell influx induced by OVA inhalation in OVA-sensitised animals or by lipopolysaccharide (LPS) exposure of non-sensitised animals. Airway function (sG_{aw}) was measured over 24 h after OVA exposure. Airway hyperresponsiveness to inhaled histamine and inflammatory cells in bronchoalveolar lavage fluid were recorded 24 h after OVA or LPS challenge. Histamine-induced bronchoconstriction was inhibited by inhaled magnesium sulphate or salbutamol alone and in combination, they produced synergistic bronchoprotection. LPS-induced neutrophil influx was inhibited by 6 days pretreatment with magnesium sulphate. Early and late asthmatic responses in OVA sensitised and challenged animals were attenuated by magnesium sulphate. Lung inflammatory cells were increased by OVA, macrophages being significantly reduced by magnesium sulphate. Nebulised magnesium sulphate protects against histamine-induced bronchoconstriction in conscious guinea-pigs and exerts anti-inflammatory activity against pulmonary inflammation induced by allergen (OVA) or LPS. These properties of magnesium sulphate explain its beneficial actions in acute asthma.

1. Introduction

The use of magnesium sulphate ($MgSO_4$) for acute asthma was first described in 1936, and since then there has been inconsistent evidence to support its use in adults and children with asthma (Mohammed and Goodacre, 2007). Recent systematic reviews have suggested that both inhaled and intravenous $MgSO_4$ has a potential role in patients with a more severe exacerbation of acute asthma (Mohammed and Goodacre, 2007; Powell et al., 2012). However, the exact role of $MgSO_4$ is not fully delineated (Rowe, 2013). A large randomized controlled trial of nebulised $MgSO_4$ with 508 children with severe acute asthma has shown a minimal effect on asthma severity scores but in those children with a more severe exacerbation and those with shorter duration of symptoms the effect appeared to be more clinically relevant (Powell et al., 2013). A randomised controlled trial with 1109 adult patients, has shown no significant clinical benefit from the addition of nebulised $MgSO_4$ to standard treatment in acute asthma, although a minimal benefit was shown in severe asthma when given intravenously (Goodacre et al., 2013).

The exact mechanism of action of $MgSO_4$ in asthma is not understood. It appears to have some bronchodilator or bronchoprotective effects for which there are a number of proposed mechanisms. *In vitro* studies demonstrate an inhibitory effect of $MgSO_4$ on contraction of bronchial smooth muscle, the release of acetylcholine from cholinergic nerve terminals, and of histamine from mast cells (Blitz et al., 2005). The main effect of $MgSO_4$ is to block the influx of calcium ions into smooth muscle cells of the airways (Georgoulianis et al., 2001) which results in bronchodilatation. One previous study demonstrates bronchoprotective effects of magnesium fluoride and $MgSO_4$ against inhaled methacholine in rats (Gandia et al., 2010), although Lindeman et al.

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http://dx.doi.org/10.1016/j.ejphar.2017.03.004
Received 13 July 2016; Received in revised form 1 March 2017; Accepted 7 March 2017
Available online 08 March 2017

(1989) failed to show any effect of intravenous $MgSO_4$ against acetylcholine-induced bronchoconstriction in anaesthetized dogs. There is evidence that $MgSO_4$ may also act as an anti-inflammatory agent by inhibiting the neutrophil respiratory burst in adults with asthma (Cairns and Kraft, 1996).

This study was undertaken to explore the actions of nebulised $MgSO_4$ in the airways of conscious guinea-pigs. There were two main objectives: Firstly, we examined whether inhaled magnesium sulphate exerts bronchodilator activity by measuring whether it has bronchoprotective properties against histamine-induced bronchoconstriction. We examined the speed of onset and dose-response relationships. Magnesium sulphate was examined on the airways function responses to inhaled histamine either alone or in combination with the β_2-adrenoceptor agonist, salbutamol. Secondly, we examined the hypothesis that inhaled magnesium sulphate inhibits airways inflammation and airways function in models of neutrophilic and eosinophilic lung inflammation. Neutrophilic inflammation was induced by inhaled lipopolysaccharide. Eosinophilic inflammation and the accompanying early and late asthmatic responses and airways hyperresponsiveness were induced by the inhaled antigen, ovalbumin, in ovalbumin-sensitised animals.

2. Materials and methods

2.1. Guinea-pig airways function and inflammation models

Naïve conscious guinea-pigs challenged with nebulised histamine exhibit an immediate bronchoconstrictor response that recovers to baseline within 10 min. Immediate bronchodilator effects of agents such as magnesium sulphate can be measured as a reduced bronchoconstriction by inhaled histamine when administered immediately before the histamine; this is known as bronchoprotection (Turner et al., 2012). To examine the effects of a drug such as magnesium sulphate against models of asthma and chronic obstructive pulmonary disease (COPD) the main features of these lung diseases need to be induced. The four main features of asthma, namely airways hyperreactivity or hyperresponsiveness (AHR), inflammatory cell influx into the airways (predominantly eosinophils) and early (EAR) and late asthmatic responses (LAR), can be reproduced in conscious guinea-pigs sensitised and challenged with ovalbumin (Toward and Broadley, 2004). Guinea-pigs challenged with inhaled lipopolysaccharide (LPS) exhibit neutrophil driven pulmonary inflammatory responses similar to COPD (Toward and Broadley, 2000). Magnesium sulphate was administered as a single dose prior to OVA challenge or as several doses over a few days before LPS challenge.

2.2. Animal husbandry

Groups of six male Dunkin-Hartley guinea-pigs (200–250 g) were obtained from Charles River, Sulzfeld, Germany and housed in solid bottomed cages. A total of 96 animals were used. Light was maintained on a 12 h cycle and food and water were available *ad libitum*. All experiments complied with the Animals (Scientific Procedures) Act, 1986 and protocols were reviewed by Cardiff University Ethical Review Panel. The ARRIVE Guidelines on Animal Research: Reporting *In Vivo* Experiments have been adhered to in the design and execution of the study (Kilkenny et al., 2012; McGrath et al., 2010).

2.3. Measurement of lung function

Airways function was measured by whole-body plethysmography as specific airway conductance (sG_{aw}) in conscious guinea-pigs using a non-invasive double-chamber plethysmograph (Buxco Systems, Wilmington, North Carolina, USA). Guinea-pigs were placed in the double chamber plethysmograph and restrained by use of a neck restraint which also separated the nasal and thoracic compartments of the chamber making them both airtight. Air temperature and gas percentages were kept constant by use of a bias flow supply unit. Box pressure changes in both compartments were measured by pressure transducers. The pre-amplified output was converted to inspiratory and expiratory waveforms by Finepoint software (Buxco system Ltd), which also derives sG_{aw}. Readings were taken every 2 s and at least 20 breaths were recorded during any time point measurement. For calculation of sG_{aw} at a particular time point, the average of 15 values taken at random was calculated.

Prior to the commencement of lung function measurements, all guinea-pigs were acclimatised to being restrained in the plethysmograph for at least 20 min on two separate occasions. This reduces movement-related signal 'noise' during measurements and reduces animal stress minimising interference from stress-related hormones such as cortisol. A decrease in sG_{aw} represents bronchoconstriction while an increase is a bronchodilator response.

2.4. Bronchoprotection by nebulised $MgSO_4$ and salbutamol

Baseline sG_{aw} readings were determined before challenge of naïve unsensitised guinea-pigs. With them still in the plethysmograph, they were challenged with nebulised histamine (0.5 mM solution for 2 min) delivered by use of a Buxco nebuliser at 0.5 l/min and 20% duty (% every 6 s of nebulising) per chamber. sG_{aw} was measured immediately after completing the challenge and at 5 and 10 min. After forty-eight hours, the same animals were treated with inhaled $MgSO_4$, salbutamol or vehicle (0.9% saline) and the histamine challenge repeated 15 min after completion of the drug or vehicle exposure. Lung function was then re-assessed as above. Each animal received one challenge with $MgSO_4$, salbutamol or vehicle in any one week.

Administration of nebulised $MgSO_4$ (62.5–250 mM), salbutamol (0.035–0.1 mM), vehicle (0.9% saline) or a combination of $MgSO_4$ and salbutamol was achieved with a DeVilbiss nebuliser (Somerset, Pennsylvania, USA) by placing the guinea-pigs in an in-house Perspex chamber (20×30×15 cm) for 15 min.

2.5. Effect of nebulised $MgSO_4$ on an ovalbumin model of asthma

Guinea-pigs were sensitised to ovalbumin on days 1, 4 and 7 by bilateral intra-peritoneal injection of ovalbumin (100 μg) and aluminium hydroxide (150 mg) suspended in saline (1 ml). On day 19, baseline sG_{aw} values were determined before placing the guinea-pigs into the Perspex exposure chamber for challenge with nebulised ovalbumin (0.03%) for 1 h using a DeVilbiss nebuliser. Guinea-pigs were removed from the chamber and sG_{aw} was measured every 15 min for the first hour following ovalbumin challenge and every hour thereafter for 12 h. A further reading was taken 24 h after the ovalbumin challenge. sG_{aw} was expressed as the percentage change from baseline. Animals were treated with nebulised $MgSO_4$ (250 mM) or vehicle for 15 min before ovalbumin challenge.

2.6. Effect of nebulised $MgSO_4$ on LPS-induced inflammation

Naïve unsensitised guinea-pigs were exposed daily to nebulised saline or $MgSO_4$ (250 mM) for 15 min in the Perspex exposure chamber (30×40×30 cm) on days 1–6. On day 4, 30 min after treatment with saline or $MgSO_4$, animals were exposed to a priming challenge with nebulised LPS (30 μg/ml) for 1 h. On day 6 they received a second identical challenge with LPS 30 min after saline or $MgSO_4$ and 24 h later, bronchoalveolar lavage was performed to determine inflammatory cell influx into the airways.

2.7. Airway responsiveness to inhaled histamine

Airway reactivity to a dose of inhaled histamine (0.3 mM) that produced non-significant threshold bronchoconstriction was evaluated 24 h before and 24 h after ovalbumin challenge. Baseline sG_{aw} values