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Mepacrine alleviates airway hyperresponsiveness and airway inflammation in a mouse model of asthma

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Abstract

Asthma is a multifactorial respiratory disease. Though its incidence is increasing rapidly all over the world, the available therapeutic strategies are neither sufficient nor safe for long term use. Mepacrine, a known antimalarial drug, has been shown to possess antioxidant, anti-inflammatory, platelet anti-aggregant, and PLA_2 inhibitory activities. However, its possible use in asthma has not been studied yet. The objective of this study was to investigate the anti-asthmatic property of mepacrine using a mouse model of asthma. To accomplish this, male BALB/c mice were sensitized and challenged with ovalbumin and treated with increasing concentrations of mepacrine. Airway hyperresponsiveness (AHR) to methacholine was assessed using unrestrained whole body plethysmography. Mepacrine (1 mg/kg) has shown marked attenuation of AHR. Cytokines such as IL-4, IL-5, IL-13 and IFN-γ and OVA-specific IgE levels were measured in BAL (bronchoalveloar lavage) fluid and sera, respectively. Mepacrine effectively reduced the rise in IL-4, IL-5, IL-13, and OVA-specific IgE and restored IFN-γ levels. Mepacrine also significantly prevented the increase of $sPLA_2$ (secretory phospholipase A_2) activity in BAL fluid supernatant and Cys-LT (cysteinyl leukotrienes) in lung tissue homogenates of asthmatic mice. In addition, mepacrine treatment reduced BAL fluid eosinophilia and signs of allergic airway inflammation such as perivascular and peribronchial distribution of inflammatory cells. These findings indicate that mepacrine reduces the asthmatic features in ovalbumin induced asthma by acting on PLA_2-Cys-LT axis. Thus, it could be useful for the development of better asthma therapy.

1. Introduction

Asthma is a multifactorial disease, characterized by various pathophysiological features such as variable degree of airway obstruction, airway hyperresponsiveness (AHR), airway inflammation, increased IgE synthesis and mucus hypersecretion [1]. Th2 cytokine response like increased IL-4, IL-5, IL-9 and IL-13 produced by T cells, mast cells and eosinophils is predominantly associated with its pathophysiology [2]. This

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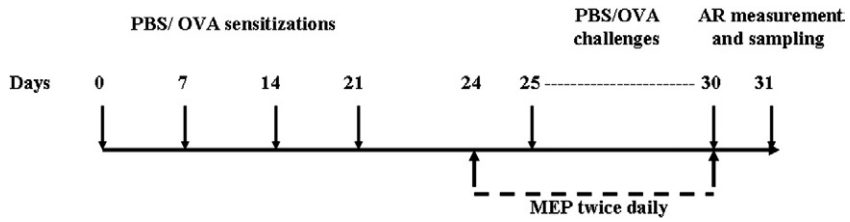

Figure 1 Schematic diagram of our experimental protocol. Mice were sensitized by intraperitoneal injections of ovalbumin (OVA) on days 0, 7, 14 and 21 and exposed to 3% OVA aerosol for 6 consecutive days (30 min daily) from day 25–30. To evaluate the effect of MEP on asthmatic parameters, mice were given mepacrine (MEP) orally from days 24 to 30. On day 31, each mouse was challenged with increasing concentrations of methacholine to determine the airway responsiveness (AR).

dominant Th2 response in the airway leads to release of numerous pro-inflammatory molecules such as lipid derived mediators, various chemokines, cytokines and growth factors from inflammatory and structural cells. Among these inflammatory mediators, lipid mediators such as cysteinyl leukotrienes have prominent role in executing the downstream effector responses like bronchoconstriction [3].

Asthma is an important public health problem world wide with significant morbidity and mortality. Though its prevalence has reached a plateau in late 1990s, it is still high in spite of major efforts taken to develop novel therapeutic strategies [4]. It could be due to either inherent complexity of the disease and/or time and financial factors in drug discovery. It has been estimated that it consumes at least 15 years and approximately £400 million to bring a new drug to a market after succeeding many phases [5]. Further, Food and Drug Administration (USA) approves only 20–30 drugs every year [5].To complicate this issue further, the existing therapy for asthma (steroids, β2-agonists and phosphodiesterase inhibitors) have intolerable adverse effects [6–9]. Hence, there is an immense need for exploring new usage for existing drugs as described elsewhere [5].

Mepacrine (6-chloro-9-(4-diethylamino-1-methylbutylamino)-methoxyacridine) is available in the market for the treatment of malaria, [10] giardiasis [11], cutaneous lupus erythematosus [12] and Creutzfeldt–Jakob diseases (CJD) [13]. Topical application of mepacrine is also well known for its non-surgical female sterilization [14]. In 1950s, a few preliminary studies indicated its usage in asthma, however there were neither any mechanistic evidence for its anti-asthma property nor any further investigations [15,16]. It has antioxidant [17], platelet anti-aggregant activities [18] other than PLA_2 inhibition [19] and it has been demonstrated that it reduces the formation of platelet activating factor which has been shown to be essential in asthma pathogenesis [20]. It has also been shown that mepacrine is able to reduce the adherence and migration of leukocyte through rat aortic endothelial cells [21]. Since oxidative stress, leukocyte recruitment and platelet activation are crucial events in the pathogenesis of asthma, we hypothesized that mepacrine could possess anti-asthmatic property. With this view, we have chosen mepacrine for evaluating its anti-asthmatic activity in a mouse model of asthma.

2. Materials and methods

2.1. Animals

BALB/c male mice (8–10 weeks old, weighing 18–20 g) were obtained from Vallabhbhai Patel Chest Institute, Delhi, India and acclimatized for 1 week under standard laboratory conditions (25 ± 2 °C, 55% humidity) before starting the experiments. Experimental protocols were approved by the Institutional Animals Ethics Committee.

2.2. Development of mouse model of asthma

To determine the effect of mepacrine (MEP) on asthmatic features, each mouse was sensitized by intraperitonial (i.p.) injections of 0.2 ml saline suspension containing 20 μg ovalbumin (OVA, grade V, Sigma, USA) and 4 mg alum (aluminum hydroxide, Sigma, USA) on days 0, 7, 14 and 21 as shown in Fig. 1. From day 25 to 30, mice were exposed to 3% OVA aerosols for 30 min/day (Fig. 1) in a plexiglas chamber ($20 \times 20 \times 10$ cm^3). The aerosol was generated by a nebulizer (Omron CX3, Japan) with an airflow rate of 9 l/min. Each control (sham) mouse received i.p. injections of 0.2 ml saline containing 4 mg alum and was challenged with saline alone.

2.3. MEP treatment to ova-sensitized and challenged mice

Mice were randomly divided into five groups (six mice in each) and each group was named according to the status of sensitization/challenge/treatment. They were SHAM/SAL/VEH (saline-sensitized, saline-challenged and vehicle treated), OVA/OVA/VEH, OVA/OVA/MEP 0.1 (mepacrine 0.1 mg/kg, in the form of mepacrine dihydrochloride, Sigma, USA), OVA/OVA/MEP 1 (mepacrine 1 mg/kg) and OVA/OVA/MEP 2 (mepacrine 2 mg/kg). Vehicle or MEP was administered orally twice daily from day 24 to 30 in the volume of 20 μl per dose. Water was used as vehicle in which MEP was dissolved.

2.4. Measurement of airway responsiveness to methacholine (MCh)

Airway responsiveness to methacholine was assessed by determining enhanced pause (Penh) using unrestrained whole body plethysmography (Buxco Electronics, NY, USA) as described earlier [22]. Briefly, each individual mouse was placed, acclimatized in whole body plethysmographic chamber and exposed to PBS aerosol to obtain baseline Penh value. Then it was exposed to aerosol of progressively doubled concentrations of methacholine (3.1, 6.25, 12.5, 25, 50 and 100 mg/ml). Airway responsiveness to MCh was evaluated by the concentration of MCh required to increase the Penh to twice the baseline value (MChPC200). This was done before sensitization (on day 0, initial MChPC200) and 24 h after last OVA or PBS challenge (on day 31, OVA or saline induced MChPC200).

2.5. Collection of bronchoalveolar lavage (BAL) fluid and blood samples

After determining airway hyperresponsiveness, mice were sacrificed, blood and BAL fluid were collected and processed to separate sera, BAL fluid supernatant and BAL fluid cells as described earlier [23]. The percentage of eosinophil count was performed in BAL fluid cells [23].