Pulmonary Pharmacology & Therapeutics 46 (2017) 30–40

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

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

# Effects of β-blockers on house dust mite-driven murine models pre- and post-development of an asthma phenotype

Radhika Joshi, Daniel Valdez, Hosu Kim, Douglas C. Eikenburg, Brian J. Knoll, Richard A. Bond*

*Department of Pharmacological and Pharmaceutical Sciences, University of Houston, 3455 Cullen Blvd., Houston, TX 77204-5027, USA*

ARTICLE INFO

*Article history:*
Received 27 March 2017
Received in revised form
16 May 2017
Accepted 15 July 2017
Available online 17 July 2017

*Keywords:*
β-adrenoceptor blockers
House dust mite
Murine model
Asthma
Extracellular-signal regulated kinases 1/2

ABSTRACT

*Background:* Our previous studies suggested certain β-adrenoceptor blockers (β-blockers) attenuate the asthma phenotype in ovalbumin driven murine models of asthma. However, the ovalbumin model has been criticized for lack of clinical relevance.
*Methods:* We tested the non-selective β-blockers, carvedilol and nadolol, in house dust mite (HDM) driven murine asthma models where drugs were administered both pre- and post-development of the asthma phenotype. We measured inflammation, mucous metaplasia, and airway hyper-responsiveness (AHR). We also measured the effects of the β-blockers on extracellular-signal regulated kinase (ERK 1/2) phosphorylation in lung homogenates.
*Results:* We show that nadolol, but not carvedilol, attenuated inflammation and mucous metaplasia, and had a moderate effect attenuating AHR. Following HDM exposure, ERK1/2 phosphorylation was elevated, but the level of phosphorylation was unaffected by β-blockers, suggesting ERK1/2 phosphorylation becomes dissociated from the asthma phenotype.
*Conclusion:* Our findings in HDM models administering drugs both pre- and post-development of the asthma phenotype are consistent with previous results using ovalbumin models and show differential effects for nadolol and carvedilol on the asthma phenotype. Lastly, our data suggest that ERK1/2 phosphorylation may be involved in development of the asthma phenotype, but may have a limited role in maintaining the phenotype.



## 1. Introduction

Asthma is a chronic disease of the airways, which occurs in people from all age groups. According to the 'Global Asthma Report', approximately 334 million people have asthma worldwide (The Global Asthma Report 2014). The numbers continue to increase making asthma a serious health and economic burden. The most often prescribed bronchodilators for asthma therapy are $\beta_2$ adrenoceptor ($\beta_2$AR) agonists. For decades, $\beta_2$AR agonists have been modified to enhance their effectiveness by improving their receptor selectivity and increasing their duration of action. However, studies have found that prolonged use of certain long acting $\beta_2$AR agonists such as salmeterol is associated with a loss of asthma control, and a small increase in asthma related deaths [1–6].

We have previously shown that mice lacking epinephrine, the endogenous agonist for the $\beta_2$AR, do not develop the asthma phenotype in ovalbumin allergen-driven models of asthma [7]. These mice lack the enzyme phenylethnolamine *N*-methyl transferase (PNMT), which is required for the final step in the synthesis of epinephrine, and have no detectable levels of circulating epinephrine [7]. In these PNMT-KO mice, chronic treatment with the long acting $\beta_2$AR agonists, salmeterol and formoterol restored the asthma phenotype [8]. Also, treatment with the β-blocker,

*Abbreviations:* AHR, airway hyperresponsiveness; $\beta_2$AR, $\beta_2$-adrenoceptor; BALF, bronchoalveolar lavage fluid; cAMP, cyclic adenosine monophosphate; ERK1/2, extracellular signal-regulated kinase 1/2; Ig, immunoglobulin; i.n., intra-nasal; i.p., intra-peritoneal; MAPK, mitogen activated protein kinase; PNMT, phenylethnolamine *N*-methyl transferase; Ova S/C, ovalbumin-sensitized and −challenged; Ova S/N, ovalbumin-sensitized and not challenged; PAFS, periodic acid fluorescent Schiff; WT, wild-type.

* Corresponding author. University of Houston, Department of Pharmacological and Pharmaceutical Sciences, Room 521, Building S&R 2, Houston, TX 77204-5037, USA.

*E-mail addresses:* rjoshi@uh.edu (R. Joshi), danvaldez2008@yahoo.com (D. Valdez), hkim36@uh.edu (H. Kim), deikenbu@central.uh.edu (D.C. Eikenburg), bknoll@central.uh.edu (B.J. Knoll), rabond@uh.edu (R.A. Bond).

http://dx.doi.org/10.1016/j.pupt.2017.07.004
1094-5539/

nadolol attenuated the asthma phenotype in wild type (WT) mice and did not restore the phenotype in PNMT-KO mice [9]. These results suggested $\beta_2$AR agonists, but not antagonists were able to restore the asthma phenotype. However, other non-selective β-blockers such as carvedilol and propranolol did restore development of the asthma phenotype in PNMT-KO mice and had no effect on the phenotype in WT mice [9].

Furthermore, pilot clinical trials have shown that chronic nadolol treatment of mild asthmatics produced a dose-dependent increase in the amount of methacholine needed to elicit a 20% reduction in forced expiratory volume in 1 s ($PC_{20}$ methacholine) [10,11], while another study showed no benefits of chronic propranolol treatment in a different set of asthmatics [12]. Thus, clinical trials also suggest mechanistic differences between different β-blockers in asthma. Several *in vitro* studies indicate that despite the ability of β-blockers to inhibit the canonical Gs-cAMP pathway at the $\beta_2$AR, they differ in their activity at the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway. In these studies, nadolol, timolol, metoprolol and ICI-118,551 inhibited the ERK1/2 pathway, while carvedilol and propranolol activated the ERK1/2 pathway [13–15]. Therefore, our *in vivo* results, and the limited data from clinical trials, suggested a correlation between the differential effects of β-blockers in the murine asthma model and their *in vitro* activation profiles at the ERK1/2 pathway [9–12]. Moreover, several studies have implicated ERK1/2 phosphorylation in the pathogenesis of asthma [16–18]. For example, administration of U0126, a mitogen activated protein kinase kinase (MEK1/2) inhibitor that inhibits ERK1/2 activation, also attenuated the asthma phenotype in an ovalbumin-driven murine asthma model [16].

However, as noted, all of our previous studies used ovalbumin as the antigen inducing the asthma phenotype. A shortcoming of ovalbumin-driven murine asthma models is their limited clinical relevance [19]. Also, persistent exposure of mice to ovalbumin could lead to immune tolerance and diminished airway inflammation [20]. To address some of these limitations, the present studies were performed using house dust mite (HDM) extract as the source of antigens. House dust mites are a common aeroallergen causing a range of respiratory symptoms in humans including asthma [21]. The whole body extract from the species, *Dermatophagoides pteronyssinus* has been used as a clinically more relevant allergen in animal models of asthma [19,21–23]. For example, a study showed that intranasal delivery of purified HDM extract for 10 consecutive days produced a robust airway inflammatory response in mice accompanied by airway hyper-responsiveness [22]. In this study, the inflammatory response resolved after 4 weeks and could be restored by subsequent re-exposure to the HDM extract [22]. Despite the differences in the allergens involved, ovalbumin and HDM driven models of asthma are qualitatively similar in terms of the phenotypes they produce in mice. To further ensure that the two models are also similar in the cytokine/chemokine profile that drives the asthma phenotypes, we also characterized the HDM model for the presence of Th1- and Th2-associated cytokines and chemokines.

In these studies we tested the β-blockers, nadolol and carvedilol in murine models of asthma utilizing house dust mite (HDM) extract. These β-blockers were chosen because as discussed above, they have opposing effects on the asthma phenotype in PNMT-KO and WT mice [9], and their activities differ at the ERK1/2 pathway in *in vitro* studies [13–15]. In addition, our previous studies had shown only a 'prophylactic' effect of β-blockers (the drugs were administered prior to the development of asthma phenotype) [9,24]. To further add clinical relevance, in the present study we investigated both the 'prophylactic' and 'therapeutic' effects (where drugs were administered after an asthma phenotype had been established) in HDM models.

Lastly, in an attempt to elucidate a potential mechanism for the differential effects of nadolol and carvedilol, we investigated ERK1/2 phophorylation in whole lung homogenates. The rationale for this was the correlation we had noted between the ability of ligands to inhibit ERK1/2, and the ability of ligands to attenuate the asthma phenotype. Additionally, previous studies implicate ERK1/2 as having a role in the development of the asthma phenotype [9,16–18]. Therefore, in the present studies, we examined ERK1/2 phosphorylation in whole lung in both the ovalbumin and the HDM-driven murine models of asthma.

## 2. Materials and methods

### 2.1. Animals

All animal experiments were performed in compliance with the ARRIVE guidelines. All procedures and protocols were approved by the Institutional Animal Care and Use Committee at the University of Houston (Protocol # 13-021, 16-022) which follows all NIH guidelines.

Male Balb/c mice (4–8 weeks old) purchased from Jackson Laboratories (Bar Harbor, ME, USA) were used in this study. Mice were housed in specific pathogen free conditions with ALPHA-dri® bedding. They were maintained at 22-24 °C and 45–48% humidity under a 12 h light/dark cycle, and were provided food and water *ad libitum*. Age-matched mice were randomly assigned to the different experimental groups.

### 2.2. Protocol timelines and drug administration

We employed house dust mite models to develop the asthma phenotypes in mice. In these models, Balb/c mice were challenged with 25 μg of HDM protein (Greer Laboratories, Lenoir, North Carolina, USA) in 10 μl sterile saline by once daily intranasal delivery according to the timelines laid out for the 'prophylactic' and 'therapeutic' protocols (Fig. 1). Three or 4 animals of the same treatment group were housed in each cage. The calculated amounts of drugs were mixed with powdered chow and filled sufficient quantities of food (~5 g per animal) in J-feeders to last a single day, and the feeders were replenished with fresh food every day.

#### 2.2.1. 'Prophylactic' model of asthma

In the 'prophylactic' model, mice were treated with β-blockers prior to development of the asthma phenotype. To establish the phenotype in the 'prophylactic' model, mice were challenged with saline or HDM protein for 5 days a week over a period of 4 weeks. During this time, groups of mice received 2400 ppm of carvedilol (Patterson Veterinary, Blythewood, SC, USA) or 250 ppm of nadolol (Sigma Aldrich, St. Louis, MO, USA) orally. As described above, the drugs were mixed with powdered rodent chow and provided to mice *ad libitum*. On day 28 of the protocol, all groups were evaluated for the various parameters of asthma.

#### 2.2.2. 'Therapeutic' model of asthma

In the 'therapeutic' model, drug treatment was started after the asthma phenotype had been established. In the 'therapeutic' model, mice were initially challenged with saline or HDM protein for 10 consecutive days. Seventy-two hours after the last challenge (protocol day 12), a control and a vehicle group were evaluated for the asthma phenotype. After the phenotype had been established, the remaining mice were started on drugs, carvedilol (2400 ppm) or nadolol (250 ppm), or vehicle administered orally in rodent chow for 4 weeks. Another vehicle group was evaluated four weeks after the last HDM challenge (protocol day 37), while the remaining vehicle and drug treatment groups were re-challenged with HDM