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Curine inhibits eosinophil activation and airway hyper-responsiveness in a mouse model of allergic asthma

Jaime Ribeiro-Filho [a,e], Andrea Surrage Calheiros [a], Adriana Vieira-de-Abreu [a], Katharinne Ingrid Moraes de Carvalho [b], Diego da Silva Mendes [a], Christianne Bandeira Melo [c], Marco Aurélio Martins [b], Celidarque da Silva Dias [d], Márcia Regina Piuvezam [e,*], Patrícia T. Bozza [a,*]

[a] Laboratório de Imunofarmacologia, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil
[b] Laboratório de Inflamação, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil
[c] Laboratório de Inflamação, Instituto Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil
[d] Laboratório de Fitoquímica, Departamento de Ciências Farmacêuticas, UFPB, João Pessoa, Paraíba, Brazil
[e] Laboratório de Imunofarmacologia, Departamento de Fisiologia e Patologia, UFPB, João Pessoa, Paraíba, Brazil

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ABSTRACT

Allergic asthma is a chronic inflammatory airway disease with increasing prevalence around the world. Current asthma therapy includes drugs that usually cause significant side effects, justifying the search for new anti-asthmatic drugs. Curine is a bisbenzylisoquinoline alkaloid that modulates calcium influx in many cell types; however, its anti-allergic and putative toxic effects remain to be elucidated. Our aim was to investigate the effects of curine on eosinophil activation and airway hyper-responsiveness (AHR) and to characterize its potential toxic effects. We used a mouse model of allergic asthma induced by sensitization and challenge with ovalbumin (OVA) to evaluate the anti-allergic effects of oral treatment with curine. The oral administration of curine significantly inhibited eosinophilic inflammation, eosinophil lipid body formation and AHR in animals challenged with OVA compared with animals in the untreated group. The curine treatment also reduced eotaxin and IL-13 production triggered by OVA. Verapamil, a calcium channel antagonist, had similar anti-allergic properties, and curine pre-treatment inhibited the calcium-induced tracheal contractile response *ex-vivo*, suggesting that the mechanism by which curine exerts its effects is through the inhibition of a calcium-dependent response. A toxicological evaluation showed that orally administered curine did not significantly alter the biochemical, hematological, behavioral and physical parameters measured in the experimental animals compared with saline-treated animals. In conclusion, curine showed anti-allergic activity through mechanisms that involve inhibition of IL-13 and eotaxin and of Ca^{++} influx, without inducing evident toxicity and as such, has the potential for the development of anti-asthmatic drugs.

Introduction

Allergic asthma is an important public health problem in terms of prevalence, morbidity and mortality, affecting approximately 20% of the world's population and requiring costly therapy (Chatila, 2004; Edwards et al., 2009). The pathophysiology of asthma is complex and results from an inappropriate immune response to common allergens; this response is characterized by chronic airway inflammation that is associated with intense leukocyte recruitment and activation at the site of injury and airway hyper-responsiveness (AHR) (Barnes, 2008; Paul and Zhu, 2010).

The current standard for the treatment of asthma involves the use of drugs that relieve and control the symptoms of the disease (Holgate and Polosa, 2008). The medications that are broadly used in asthma management today include potent inhaled corticosteroids, such as budesonide and fluticasone; long-acting β_2-adrenergic agonists (LABAs), such as salmeterol and formoterol; and leukotriene modifiers, including zafirlukast, montelukast and zileuton; there are also combination therapies that include inhaled corticosteroids and LABAs in a single delivery device (Szefler, 2011). Corticosteroids are potent anti-inflammatory drugs that regulate the expression of cytokines, chemokines and adhesion molecules by modulating the activity of transcription factors such as nuclear factor-κB (NF-κB) and activator protein 1 (AP1). Inhaled corticosteroids are very effective at inhibiting airway inflammation, and they represent an important tool in asthma management (Ivancsó et al., 2013). However, these drugs are not effective under specific conditions, including virus- or smoke-induced exacerbations (Harrison et al., 2004). β_2-Adrenergic agonists effectively promote bronchodilation through the production of cyclic adenosine 3′5′-monophosphate (cAMP) and the activation of protein kinase A (PKA). Inhaled short- and long-acting β_2-adrenergic agonists are

* Corresponding authors.
E-mail addresses: mrpiuvezam@ltf.ufpb.br (M.R. Piuvezam), pbozza@ioc.fiocruz.br (P.T. Bozza).

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currently most commonly used in association with inhaled corticosteroids as a supplementary therapy for asthma (Holgate and Polosa, 2008). Phosphodiesterase inhibitors also increase cAMP, but these treatments have been less commonly used of late because of the low therapeutic index of some drugs (Boswell-Smith et al., 2006). Antagonists of cysteinyl leukotriene receptor 1 (CysLTR1) are currently available and are important therapeutic tools in asthma. These drugs act by blocking many activities of CysLTs, including bronchoconstriction, and are mainly used as a supplementary therapy to inhaled corticosteroids (Polosa, 2007). Finally, mast cell inhibitors, cytokine-blocking monoclonal antibodies and allergen-specific immunotherapies are also important in the treatment of asthma and other allergic diseases (Edwards and Howell, 2000; Holgate and Polosa, 2008).

Despite the currently available array of anti-asthmatic therapies, the search for structurally novel chemicals and for the development of safe and effective drugs for the treatment of asthma and other allergic conditions remains an important field of investigation. Several studies have shown that bisbenzylisoquinoline alkaloids (BBAs) have immunomodulatory activity against allergy and inflammation (Seow et al., 1986; Teh et al., 1990). Our group has demonstrated that the alkaloid warifteine has anti-allergic properties, including the inhibition of anaphylaxis, the inhibition of eosinophil recruitment and activation, the ability to modulate airway hyper-responsiveness and remodeling (Bezerra-Santos et al., 2005, 2006, 2012) and the ability to inhibit histamine release (Costa et al., 2008).

Curine (Fig. 1A) is a BBA that is the major constituent of the root bark of *Chondrodendron platyphyllum* (Menispermaceae). This compound has a molecular structure that is similar to that of warifteine. Recently, Medeiros et al. (2011) demonstrated that curine may have direct effects on L-type Ca^{2+} channels in vascular smooth muscle cells. However, despite curine's interesting pharmacological activity and its structural similarity to warifteine, this molecule's anti-allergic properties and potential for toxic effects remain to be investigated. Using a mouse model of allergic asthma and finding no detectable toxicity, our study is the first to report that curine is beneficial as an orally active anti-allergic compound.

Methods

Curine purification. *C. platyphyllum* Hil St. (Miers) was collected in the municipality of Santa Rita, Paraíba, Brazil. The voucher specimen of this plant is deposited in the Herbarium Prof. Lauro Pires Xavier, number 3631-P, and was identified by Prof. Dr Maria de Fatima Agra. Spectroscopically pure curine was isolated from the root bark of *C. platyphyllum* as described by Mambu et al. (2000). The curine solution was prepared using 1 mg of the crystal in 50 μl of 1 N HCl and 500 μl of distilled water.

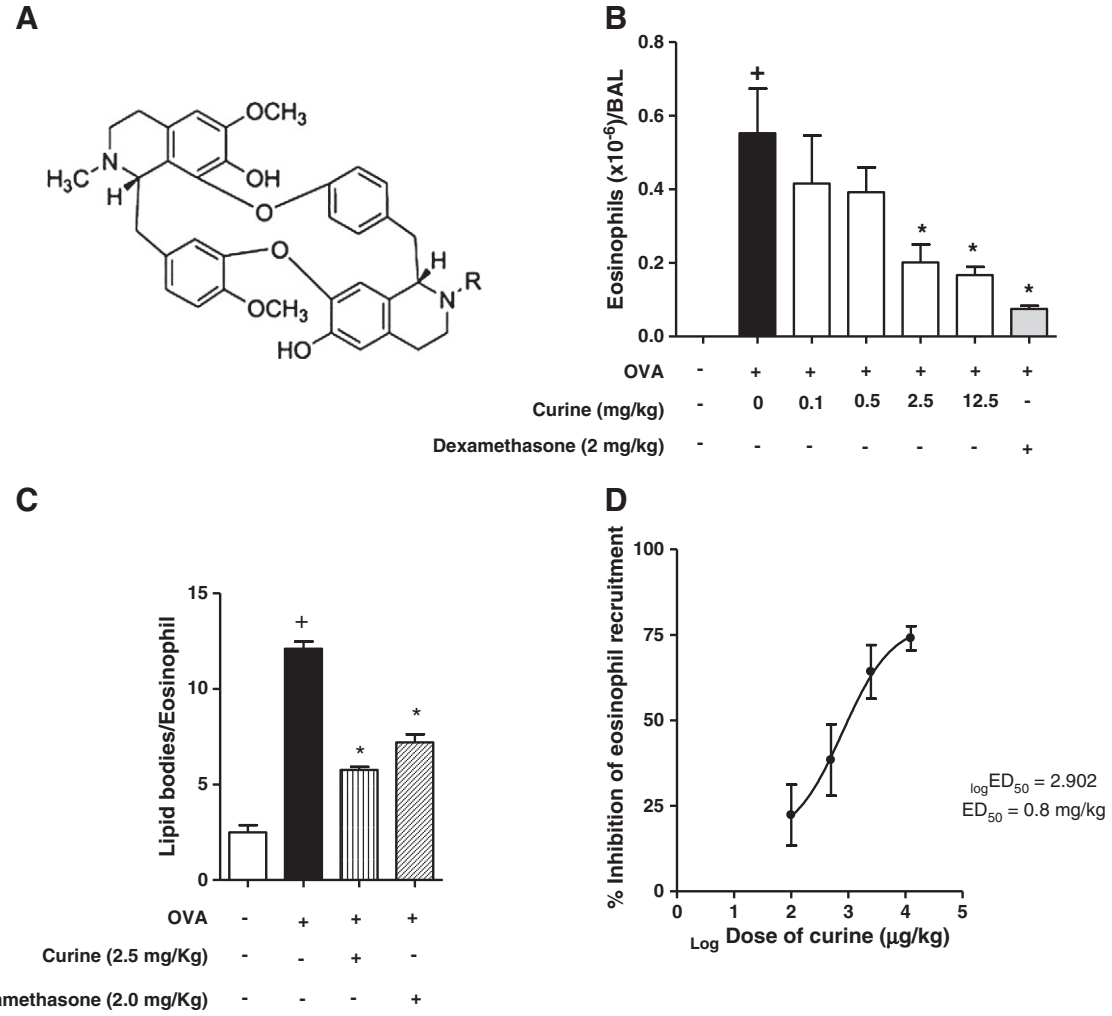

Fig. 1. The effect of curine on eosinophil recruitment and activation. BALB/c mice (n = 5–7) were treated orally with increasing doses of curine (0.1, 0.5, 2.5 or 12.5 mg/Kg), or dexamethasone (2 mg/Kg) 1 h before each challenge. Twenty-four hours after the last challenge, the BAL was collected and the eosinophils and lipid bodies were counted using a light microscope. A) the chemical structure of curine; B) the number of BAL eosinophils; C) the number of lipid bodies per eosinophil; D) a dose–response curve of curine on the inhibition of eosinophil recruitment. These results are expressed as the mean ± SEM of at least 5 animals. + Significantly different ($p < 0.05$) from the unchallenged group; * significantly different from the untreated, OVA-challenged group.