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Research Paper

Curine inhibits mast cell-dependent responses in mice

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ABSTRACT

Ethnopharmacological relevance: Curine is a bisbenzylisoquinoline alkaloid and the major constituent isolated from *Chondrodendron platyphyllum*, a plant that is used to treat inflammatory diseases in Brazilian folk medicine. This study investigates the effectiveness of curine on mast cell-dependent responses in mice.

Materials and methods: To induce mast cell-dependent responses, Swiss mice were subcutaneously sensitized with ovalbumin (OVA—12 μg/mouse) and $Al(OH)_3$ in a 0.9% NaCl solution. Fifteen days later, the animals were challenged with OVA through different pathways. Alternatively, the animals were injected with compound 48/80 or histamine, and several parameters, including anaphylaxis, itching, edema and inflammatory mediator production, were analyzed. Promethazine, cromoglycate, and verapamil were used as control drugs, and all of the treatments were performed 1 h before the challenges.

Results: Curine pre-treatment significantly inhibited the scratching behavior and the paw edema induced by either compound 48/80 or OVA, and this protective effect was comparable in magnitude with those associated with treatment with either cromoglycate or verapamil. In contrast, curine was a weak inhibitor of histamine-induced paw edema, which was completely inhibited by promethazine. Curine and verapamil significantly inhibited pleural protein extravasations and prostaglandin D_2 (PGD_2) and cysteinyl leukotrienes (CysLTs) production following allergen-induced pleurisy. Furthermore, like verapamil, curine inhibited the anaphylactic shock caused by either compound 48/80 or an allergen. In *in vitro* settings, these treatments also inhibited degranulation as well as PGD_2 and CysLT production through IgE-dependent activation of the mast cell lineage RBL-2H3.

Conclusion: Curine significantly inhibited immediate allergic reactions through mechanisms more related to mast cell stabilization and activation inhibition than interference with the pro-inflammatory effects of mast cell products. These findings are in line with the hypothesis that the alkaloid curine may be beneficial for the treatment of allergic disorders.

1. Introduction

Allergic diseases are an important public health problem, affecting approximately 20% of the world population (Edwards et al., 2009). The most common allergic diseases include asthma, rhinoconjunctivitis, sinusitis, food allergy, atopic dermatitis, angioedema, urticaria, anaphylaxis and allergy to drugs and insects (Holgate and Polosa, 2008). The allergic reactions are triggered by stimuli that lead to mast cell activation, causing degranulation; the release of pre-formed mediators, such as histamine and serotonin; and the synthesis of cytokines and lipid mediators, including prostaglandin D_2 (PGD_2) and cysteinil leukotrienes (CysLTs) through specific signaling pathways (Cockcroft et al., 2007; Gould and Sutton, 2008). Under allergic conditions, mast cell activation is triggered by the IgE–FcεRI complex (Galli et al., 1999; Gould and Sutton, 2008). It has been demonstrated that

Abbreviations: AHR, airway hyper-responsiveness; BBA, bisbenzylisoquinoline alkaloid; β-hex, beta-hexosaminidase; COX, cyclooxygenase; CysLT, cysteinyl leukotriene; DNP, dinitrophenol; IgE, Immunoglobulin E; IL-(), interleukin-(); OVA, ovalbumin; PG (), prostaglandin

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mast cells can be activated by synthetic compounds, such as calcium channel agonists and compound 48/80, which is an important experimental tool in allergy research (Tatemoto et al., 2006). Because histamine is a major mediator in immediate allergic responses, histamine receptor antagonists and mast cell stabilizers have been used to treat many allergic symptoms, including edema and itch. However, there are numerous conditions for which they are not effective (Cook et al., 2002; Kim, 2012), justifying the search for substances that can be used as molecular templates in drug discovery for allergy.

Curine is a bisbenzylisoquinoline alkaloid (BBA) and the major constituent of the root bark of *Chondrodendron platyphyllum* (Menispermaceae). This plant is popularly known as "abútua" and has been used in Brazilian folk medicine to treat inflammatory conditions (Correa, 1984; Gotfredsen, 2013). Earlier reports demonstrated that curine has vasodilator effects associated with the inhibition of calcium influx, possibly through a direct blockade of L-type Ca^{2+} channels (Dias et al., 2002; Medeiros et al., 2011).

We have demonstrated the anti-allergic properties of curine administered orally using a mouse model of allergic asthma. In these experiments, curine significantly inhibited eosinophilic inflammation, eosinophil lipid body formation, cytokine production and airway hyper-responsiveness (AHR) *in vivo*. 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. Importantly, oral treatment with curine for 7 consecutive days in doses up to 10-fold higher than its median effective dose (ED_{50}) did not induce evident toxicity (Ribeiro-Filho et al., 2013).

Additionally, we have demonstrated that curine has anti-inflammatory and analgesic effects due to its inhibition of edema and vascular permeability and its inhibition of the nociceptive responses associated with an anti-inflammatory but without neurogenic mechanisms. Importantly, curine inhibited PGE_2 production *in vitro*, without affecting COX-2 expression. Moreover, the effects of curine treatment were similar to those of indomethacin (a non-steroidal anti-inflammatory drug) but were different from morphine (a central acting analgesic drug), suggesting that the analgesic effects of curine do not result from a direct inhibitory effect on neuronal activation but that they depend on anti-inflammatory mechanisms that, at least in part, result from the inhibition of PGE_2 production (Leite et al., 2014).

Despite the consistent anti-inflammatory and anti-allergic properties of curine, the effects of this compound on mast cell-dependent responses remain to be elucidated. Therefore, this work aims to characterize the effects of curine on mast cell-dependent responses in mice.

2. Methods

2.1. Curine purification

Chondrodendron platyphyllum Hill St. (Miers) was collected in Santa Rita, Paraíba, Brazil. The specimen voucher was deposited in the Herbarium of Prof. Lauro Pires Xavier (UFPB - João Pessoa, Brazil), number 3631-P, and identified by Prof. Dr. Maria de Fatima Agra. The *Chondrodendron platyphyllum* bark was dried and pulverized in a HARLEY type grinder and then extracted under exhaustive percolation with 95% ethanol for 3–4 days. The extract was concentrated under vacuum at temperatures ranging from 50°C to 60°C to obtain the crude ethanol extract. This extract was then dissolved in 3% HCl, filtered through Celite (545 Fischer Scientific) and submitted to $CHCl_3$ extraction alternated with NH_4OH (pH 8) basification. After washing with water and $MgSO_4$, the solvent was evaporated, and this $CHCl_3$ extract became the total tertiary alkaloid fraction (TTA). The TTA was separated using column chromatography ($60 \times 600\ mm^2$) on aluminum oxide using a step gradient of hexane, hexane-CH_2Cl_2 and CH_2Cl_2:MeOH. Fractions of 50 mL were collected and monitored using TLC with a step gradient of $CHCl_3$–MeOH 100:0 (3 L) and 97:3 (2 L). Fractions of 50 mL were collected for each system (hexane (100%), hexane: CH_2Cl_2 (1:1), CH_2Cl_2 (100%), CH_2Cl_2:MeOH (99:1), CH_2Cl_2:MeOH (8.5:1.5) and CH_2Cl_2:MeOH (2.5:7.5)), yielding 60 fractions. Fractions 52–60 monitored using TLC (system 6) contained curine (0.031% of dry plant; purity > 98%) that achieved fusion at 215 °C $[\alpha]_D$–225°C (MeOH, $c = 0.04$). The structure was established using spectroscopic NMR ^{13}C and NMR ^{1}H ($CDCl_3$, 400 MHz) data analysis (Leite et al., 2014), which demonstrated that the product was curine when compared to the literature data (Mambu et al., 2000). The curine solution was prepared using 1 mg of crystalline powder, 50 μL of 1 N HCl and 500 μL of distilled water. The pH was adjusted to 7–8 with 1 N NaOH. The volume was brought up to 1 mL with phosphate buffered saline (PBS).

2.2. Animals

Male Swiss mice weighing 25–30 g were obtained from the breeding units at the Oswaldo Cruz Foundation and Federal University of Paraiba. The animals were maintained with food and water *ad libitum* in a room with a temperature ranging from 22 to 24 °C and a 12 h light/dark cycle. This study was carried out in accordance with the recommendations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA). The protocols were approved by the Animal Welfare Committee of the Oswaldo Cruz Foundation (CEUA/FIOCRUZ protocol # L-002/08) and by the Ethical Committee for Experimental Animal (CEPA No. 0504/08, UFPB). Groups of 6–10 animals were used in each experiment.

2.3. Treatments

For the *in vivo* experiments, the animals were orally (p.o.) or subcutaneously (s.c.) pre-treated with curine (2.5 mg/kg). Alternatively, the groups of mice were treated with either an intraperitoneal (i.p.) injection of sodium cromoglycate (10 mg/kg), an intramuscular (i.m.) injection of promethazine (10 mg/kg) or an oral (p.o.) administration of verapamil (2.5 mg/kg), all of which were used as reference drugs. PBS (p.o.) was given as a negative control. All treatments were performed 1 h before the allergic challenge. For the *in vitro* experiments, RBL-2H3 cells were exposed to curine (1 or 10 μM) or vehicle 1 h before the stimulus. The dose of curine (2.5 mg/kg) was chosen based on the data obtained from a prior study (Ribeiro-Filho et al., 2013). Of note, the curine concentrations used in the *in vivo* experiments did not affect cell viability (viability > 95%).

2.4. Induction and evaluation of the scratching behavior (itching)

This protocol was used to evaluate the potential of curine in modulating allergic itching. For this purpose, Swiss mice were treated subcutaneously (s.c.) with curine. One hour later, the animals received an intradermal injection of compound 48/80 (10 μg) dissolved in PBS (20 μL) in the rostral part of the back. Immediately after the injection, the animals were placed under observation in individual boxes and their behavior was monitored for 60 min. The results are expressed as the number of times that the animals scratched the injection site with the hind paw over 60 min (number of scratchings) (Inagaki et al., 2002).