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Androctonus australis hector venom contributes to the interaction between neuropeptides and mast cells in pulmonary hyperresponsiveness

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ABSTRACT

Lung injury and respiratory distress syndrome are frequent symptoms observed in the most severe cases of scorpion envenomation. The uncontrolled transmigration of leukocyte cells into the lung interstitium and alveolar space and pulmonary edema may be the cause of death. Mast cells can release various inflammatory mediators known to be involved in the development of lung edema following scorpion venom injection. The present study was designed to determine the evidence of neurokinin 1 (NK1) receptor and the involvement of mast cell activation to induce pulmonary edema and to increase vascular permeability after *Androctonus australis hector* (*Aah*) venom administration. To this end, mast cells were depleted using compound 48/80 (C48/80). Furthermore, the involvement of tachykinin NK1 receptors expressed on mast cell membranes was elucidated by their blocking with an antagonist. On the other hand, the ability of *Aah* venom to increase vascular permeability and to induce edema was also assessed by measuring the amount of Evans blue dye (EBD) extravasation in bronchoalveolar lavage (BAL) fluid and in the lungs of mice. Pulmonary edema, as assessed by the levels of EBD extravasation, was completely inhibited in compound 48/80-treated animals. Depletion by stimuli non-immunological C48/80 component markedly reduced induced inflammatory response following the venom administration. The mast cells seem to play an important role in the development of lung injury and the increase of vascular permeability in mice following the subcutaneous administration of *Aah* scorpion venom through the NK1 receptor.

1. Introduction

Scorpion envenomation is widespread in several countries, where it still constitutes a significant public health problem [1]. Various symptoms, including pain and cardiovascular and respiratory disturbances, are observed when animals or humans are stung by scorpions. In the most severe cases of envenomation, pulmonary edema is an important finding and commonly the cause of death [2]. It has been attributed to acute left ventricular failure resulting from massive catecholamine release or myocardial damage induced by the venom [3,4].

In Algeria, *Androctonus australis hector* (*Aah*) is mainly involved in serious and fatal events especially after children envenoming [5]. The bioactive substances of scorpion venom are neurotoxic peptides present in small amounts (<5% of venom dry weight), they are responsible for almost all fatal cases in mammals. The toxicity of *Aah* venom is mainly due to low molecular weight (~7 kDa) neurotoxins that act on the voltage-gated sodium channels of excitable cells [1]. *Aah* venom appears to be related to an increase of pulmonary vascular permeability, accompanied by activation of the inflammatory cascade with severe damage in lung tissue, such as diffuse injury in the alveolar capillary barrier and interstitial edema with marked leukocyte infiltration [6–8]. The lethality has been attributed to severe cardio-respiratory, metabolic and neurological abnormalities, destabilizing the neuro-immuno-endocrinal axis, explained by the ability of toxins to act on sodium channels on neuronal terminals, leading to depolarization of axonal membranes and consequent release of neuromediators which will stimulate various organs including the gut, heart and vascular tissue [9–12]. Pulmonary edema is a frequent symptom in the most severe cases of envenomation, it is characterized by altered lung function and increase in pulmonary vascular permeability. The cross-talk between immune and nervous system are involved in cardiac arrhythmias, arterial hypertension followed by hypotension, marked increase in blood pressure associated with left ventricular failure, acute pulmonary edema, cardio-respiratory disturbances and biological abnormalities such as leukocytosis and metabolical perturbation [7,8,13–17]. The activation of the inflammatory signaling cascade and the release of lipid-derived mediators of inflammation may also be involved in the development of cardio-respiratory disorders [14,18–20]. Pulmonary edema, disruption of myocardial fibers and alveolar and myocardial hemorrhages were frequently described in previous studies [21–26]. Many structural changes occur in reactive lungs, including epithelial shedding, enlarged

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submucosal glands, subepithelial basement membrane thickening and fibrosis, as well as increased smooth muscle, which increases in amount by hyperplasia and hypertrophy, contributing to airway wall thickness which is also driven by deposition of extra cellular matrix including collagen [27–30].

Mast cells are long-lived cells that can proliferate locally when stimulated appropriately [31]. These cells are widely distributed throughout the body and their characteristics differ depending on their location, particularly near surfaces exposed to the environment [32]. Mast cells can be activated to secrete diverse mediators and cytokines by immunoglobulin E (IgE) and specific antigen and many other stimuli. The mast cell has been known to be a key effectors cell type in IgE-mediated immediate hypersensitivity and allergic disorders. Although, further studies have added their novel roles in host defense against bacteria and bee and snake venoms, as well as immune regulatory effects. Mast cells have been shown to be increased in allergy and asthma [33–35]. Mast cells are activated by cross-linking of IgE-bound high-affinity IgE receptors (FcεRI) with multivalent antigen that leads to downstream signal transduction and induces the allergic response. After cell activation, the preformed pro-inflammatory mediators are secreted, such as histamine, serotonin and proteases among other inflammatory mediators, including cytokines, chemokines, leukotrienes and prostaglandins [36]. It has also been demonstrated that monomeric IgE binding to FcεRI elicits numerous biological activities in mast cells: upregulated cell surface expression of FcεRI, increased histamine and leukotriene release, receptor internalization, DNA synthesis, increased filamentous actin content, membrane ruffling, adhesion to fibronectin and migration [37,38]. Many of the mast cell products described above as mediators of normal physiological processes, if released in excess, can trigger allergic and anaphylactic responses, and the inflammation is typically Th2 driven involving many of the mediators [39]. On the other hand, mast cells are also activated by the tachykinin substance P (SP) *via* distinct mechanisms. First, SP can activate mast cells without an intermediary receptor through direct combination with G proteins on the cell surface. Second, tachykinins interact with specific membrane proteins belonging to the family of G protein-coupling cell membrane receptors. These receptors are linked to various physiological and biological effects such as regulation of neurotransmission, pain, inflammation, cell growth and differentiation and oncogenesis [40]. SP and the subsequent activation of the NK1 receptor lead to phosphoinositide hydrolysis, calcium mobilization and mitogen-activated protein kinase (MAPK) activation [41]. The increased of NK1 receptor expression was reported in inflamed tissue [42]. Therefore, it can be proposed that NK1 receptor expression on immune cells, such as mast cells, could be influenced by environmental inflammatory factors such as cytokines including SP signaling. It was shown that SP activation is initiated only after interaction between nerve fibers and associated basophil leukemia cell line (RBL) cells through NK1 receptors [43]. The extent of degranulation of bone marrow-derived mast cells (BMMCs) in mice depends directly on both the concentration of SP used and the amount of NK1 receptor expression.

We have previously demonstrated that lung edema induced after envenoming by *Aah* scorpion venom was due to the activation of the inflammatory cascade and release of lipid-derived mediators of inflammation [7,44]. However, it is still unclear how *Aah* venom could activate the inflammatory cascade and, hence, induce pulmonary edema. One distinct possibility was raised from the involvement of mast cells in the lung edema induced by scorpion venoms [45–47]. Mast cells are also involved in inflammatory responses reaching different organs induced by other types of venoms, such as snake, bee, spider or wasp venoms [48–53]. Furthermore, from the important contribution of neuropeptides, in particular substance P to activate mast cells to release inflammatory mediators, such as leukotriene, PAF (platelets activating factor) and prostaglandins, and the crucial role of tachykinin NK1 receptor antagonists in the lung injury induced by *Tityus serrulatus* scorpion venom [54,55].

The present work was designed to investigate whether mast cells participated in the acute lung injury induced by *Aah* scorpion venom and could, thus, be an intermediate between neuropeptide release and activation of the inflammatory cascade including neurokinin receptor NK1.

2. Materials and methods

2.1. Venom

Crude *Aah* venom was provided from Pasteur Institute of Algeria. It was supplied in lyophilized form and stored at 4 °C. The lethal dose (LD50) of *Aah* venom is estimated to be 0.85 mg/kg by intraperitoneal (i.p.) route [56]. *Aah* venom toxic fraction (FtoxG-50) and non-toxic fraction (F1) were isolated from the venom by gel filtration through Sephadex G50 column as previously reported. The toxins contained in the FtoxG-50 account for the almost totality of the venom toxicity with LD50 of 0.65 mg/kg [56].

2.2. Animals

Adult male NMRI mice (*Mus musculus*) with an average weight of 20 ± 2 g were purchased from the animal breeding of the Pasteur Institute of Algeria. They were housed in temperature and humidity-controlled rooms and received food and water *ad libitum* before being used for study. The experiments were achieved in line with the current guidelines for the care of laboratory animals.

2.3. Reagents

All used chemicals and reagents were of highest quality commercially available. LY303870 has been obtained such as a gift. Evans blue dye, dimethylformamide, compound 48/80, May–Grunwald–Giemsa, *o*-phenylenediamine (OPD), hydrogen peroxide (H_2O_2), Tris hydrochloride, Triton X-100, sulfuric acid (H_2SO_4), hexadecyltrimethylammonium bromide (HTAB), o-dianisidine dihydrochloride, formaldehyde, paraffin, acetic acid, anti-IgE antibody, Tween-20, toluidine blue, hematoxylin and eosin were purchased from Sigma-Aldrich Chemical Co., St Louis, MO, USA.

2.4. Animals and experimental protocol

The animals were divided into four groups of 6 mice each. The first group, used as control, was injected subcutaneously with 100 μL of physiological saline solution (0.9% NaCl). The second group received a subcutaneous (s.c.) injection of sublethal dose (10 μg/20 g body weight) of *Aah* venom. The third group was injected subcutaneously with a sublethal dose (8 μg/20 g body weight) of FtoxG-50 fraction, and the fourth one with 8 μg/20 g body weight of F1 fraction. Blood samples, lungs, bronchoalveolar lavage (BAL) and peritoneal liquid (PL) were collected at different time intervals (1, 2, 4, 6 and 24 h) following the experimental envenomation. This experimental protocol was the same applied with pretreatments described below.

2.5. Reduction of mast cell function by depletion with compound 48/80

The method used has been already described [57,58]. The mice were intraperitoneally pretreated with C48/80 for 4 days at doses known to induce degranulation and depletion of mast cells. A dose of 0.6 mg/kg of C48/80 was administered twice a day for 3 days and 1.2 mg/kg twice on the fourth day, experiments were performed on the next day by injecting the whole venom, FtoxG-50 fraction or F1 fraction as described above. The depletion of the mast cell population was estimated by counting the number of mast cells present in the peritoneal cavity after 4 h. Total leukocyte counts in BAL fluid were performed by a hemocytometer (Hema-screen 13, Hospitex Diagnostics) followed