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Immunopathologic effects of scorpion venom on hepato-renal tissues: Involvement of lipid derived inflammatory mediators

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ABSTRACT

Scorpion venoms are known to cause different inflammatory disorders through complex mechanisms in various tissues. In the study here, the involvement of phospholipase A_2 (PLA_2) and cyclo-oxygenase (COX)-derived metabolites in hepatic and renal inflammation responses were examined. Mice were envenomed with *Androctonus australis hector* scorpion venom in the absence or presence of inhibitors that can interfere with lipid inflammatory mediator synthesis, i.e., dexamethasone (PLA_2 inhibitor), indomethacin (non-selective COX-1/COX-2 inhibitor), or celecoxib (selective COX-2 inhibitor). The inflammatory response was assessed by evaluating vascular permeability changes, inflammatory cell infiltration, oxidative/nitrosative stress marker levels, and by histologic and functional analyses of the liver and kidney. Results revealed that the venom alone induced an inflammatory response in this tissues marked by increased microvascular permeability and inflammatory cell infiltration, increases in levels of nitric oxide and lipid peroxidation, and decreases in antioxidant defense. Moreover, significant alterations in the histological architecture of these organs were associated with increased serum levels of some metabolic enzymes, as well as urea and uric acid. Pre-treatment of mice with dexamethasone led to significant decreases of the inflammatory disorders in the hepatic parenchyma; celecoxib pre-treatment seemed to be more effective against renal inflammation. Indomethacin pre-treatment only slightly reduced the inflammatory disorders in the tissues. These results suggest that the induced inflammation response in liver was mediated mainly by PLA_2 activation, while the renal inflammatory process was mediated by prostaglandin formation by COX-2. These findings provide additional insight toward the understanding of activated pathways and related mechanisms involved in scorpion envenoming syndrome.

1. Introduction

Several cases of scorpion envenomation are reported yearly in tropical and subtropical countries (Chippaux and Goyffon, 2008). The lethality of scorpion venoms has been mainly associated with their neurotoxins' ability to modify functional properties of ion channels in excitable tissues. The concomitant massive release of neurotransmitters and repetitive neuronal depolarizations result in various symptoms such as pain, sweating, fever, and hypertension (Amitai, 1998; Ismail, 1995; Quintero-Hernandez et al., 2013). In more severe cases, these symptoms are associated with cardiac failure, pulmonary edema, and shock (Aboumaad et al., 2014; Adi-Bessalem et al., 2008; Bahloul et al., 2013; De Matos et al., 1997; Sami-Merah et al., 2008; Yarom et al., 1970). The liver and kidney are also targeted by scorpion venoms, with the main lesions in the resulting hepatotoxicity/nephron-toxicity being reported are edema, necrosis, hemorrhage, and inflammatory cell infiltration (Adi-Bessalem and Laraba-Djebari, 2013; Al-Harbi and Al-Hasawi, 2014; Bessalem et al., 2003; Boussag-Abib and Laraba-Djebari, 2011; D'Suze et al., 2004).

Several mediators are involved in the immuno-inflammatory processes that play a key role in biological disorders/tissue damage induced by scorpion envenomation (Petricevich, 2010). The exact mechanism of triggering is multi-factorial and related mainly to generation of several bioactive molecules, such as cytokines, histamine, kinins, and lipid mediators derived from arachidonic acid (Adi-Bessalem and Laraba-Djebari, 2013; Fukuhara et al., 2003; Lamraoui et al., 2014; Liu et al., 2007; Pessini et al., 2008; Zoccal et al., 2013).

Prostaglandins are one of bioactive lipids that play an important role in a number of biological functions including cell division and immune responses (Herschman, 1996). Cyclo-oxygenase (COX) is the key enzyme in the synthesis of prostaglandins from arachidonic acid, obtained by phospholipase A_2 (PLA_2) on membrane phospholipids. COX converts arachidonic acid to PGH_2, a common precursor of all prostanoids. Two

Abbreviations: Aah, *Androctonus australis hector* scorpion; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CEL, celecoxib; COX, cyclooxygenase; DEX, dexamethasone; EPO, Eosinophile peroxidase; GSH, glutathione; ICAM-1, Inter Cellular Adhesion Molecule; INDO, indomethacin; IL-8, interleukin 8; IL-6, interleukin 6; MDA, malondialdehyde; MPO, myeloperoxidase; NF-kB, nuclear factor-Kb; NMRI, naval Medical Research Institute; NO, nitric oxide; PGE_2, prostaglandin E_2; PGH_2, prostaglandin H_2; PLA2, phospholipase A2; TBA, thiobarbituric acid.

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isoforms of COX have been described, cyclooxygenase 1 (COX-1) and 2 (COX-2). COX-1 is constitutively expressed whereas COX-2 is induced by pro-inflammatory stimuli and generates an over-production of pro-inflammatory prostaglandins (Simmons et al., 2004; Wang and Dubois, 2010). Induction of COX-2 in immune cells, as well as in other cell types (like fibroblasts and endothelial cells) is thought to be important for prostaglandin production in inflammatory processes (Akarasereenont et al., 2002; Fogel-Petrovic et al., 2004; Gilroy et al., 2001). These mediators are involved in promoting vasodilatation and local attraction and activation of inflammatory cells at early stages of inflammation (Neisius et al., 2002; Tajima et al., 2008; Weller et al., 2007; Yu and Chadee, 1998).

Among the principle bioactive prostaglandins derived from COX-2, PGE_2 could be involved in the inflammatory process induced by scorpion envenomation and to have a role in neutrophil requirement (Pessini et al., 2006; Zoccal et al., 2013). Until now, no studies have looked at the role of lipid-derived inflammatory mediators in hepatic and renal inflammation and inflammatory pathways involved in production of these mediators in scorpion envenomation. Therefore, the present study sought to evaluate the role of PLA_2 and COX pathways in the induced hepato-renal inflammatory response.

2. Materials and methods

2.1. Venom

Androctonus australis hector (Aah) scorpion venom was supplied in lyophilized form from the Laboratory of Cellular and Molecular Biology of the Biological Sciences Faculty at USTHB (Algiers, Algeria). While a lethal dose (LD_{50}) of the venom in mice was previously estimated to be 0.85 mg/kg by the intraperitoneal (IP) route (Laraba-Djebari and Hammoudi, 1998), our other studies using injection of the venom by the subcutaneous route (which mimics accidental stings in nature) showed that a dose of 0.5 mg/kg caused a mild envenomation without death (Adi-Bessalem et al., 2008, 2012a; Chaïr-Yousfi et al., 2015; Lamraoui et al., 2014).

2.2. Animals

NMRI (Naval Medical Research Institute) Swiss mice (male, 20–22 g, 6–8-wk.-of-age) were obtained from the animal breeding division of the Biological Sciences faculty of the USTHB. All mice were housed under controlled conditions of humidity, lighting, and temperature (50 ± 10% RH, 12-h light/dark, 23 ± 2 °C) throughout the experiments, and had ad libitum access to standard rodent chow and filtered water. All animal experiments were planned and performed in accordance with guidelines for the care of laboratory animals and approved by the European community Council Directive (86/609/EEC).

2.3. Reagents

The chemicals and reagents used were of analytical high quality and they were mostly from Sigma (St. Louis, MO) and Merck (Mannheim, Germany). The drugs used in this study, i.e., dexamethasone, indomethacin, and celecoxib, were purchased from, respectively, Laboratorio Farmacologico Milanese SRL (Milan, Italy), Pfizer (Berlin, Germany), and LADPharma (Algiers, Algeria).

2.4. In vivo protocols

Mice were randomly allocated into five groups (n = 9/group). Mice in the control group were to receive a subcutaneous injection of saline vehicle. Mice in Group 2 were to be injected with a sublethal dose of Aah venom (0.5 mg/kg, SC). Various drugs that can interfere with synthesis of lipid-derived metabolites were used prior to the injection of venom. Mice were to be pre-treated with (1) dexamethasone (to sup-press phospholipase A_2 activity) at 5 mg/kg IP 30 min before venom (Group 3), (2) indomethacin (non-select inhibitor of COX-1 and COX-2) at 2 mg/kg intravenous (IV) 30 min before venom (Group 4), or (3) celecoxib (selective inhibitor of COX-2) at 10 mg/kg IP 1 h before the venom (Group 5). After 24 h, all mice were euthanized by CO_2 inhalation. At necropsy, blood and organs (liver and kidney) were collected, weighed, and then processed for further analysis in the assays outlined below (Fig. 1).

2.5. Determination of vascular permeability changes

Vascular permeability was measured in different tissues by quantification of Evans Blue extravasation (De Matos et al., 1999). This marker dye was injected to mice intravenously (20 mg/kg) a few seconds before injection of the saline or venom. From the tissues collected at 24 h post-treatment, samples of the tissues were retrieved, weighed, and placed in 2 ml formamide. After incubation at 37 °C for 48 h, the absorbance of the Evans blue dye was measured at 620 nm in an ULTROSPEC 1100 PRO UV/Vis spectrophotometer (Amersham Biosciences, Buckinghamshire, UK). The concentration of Evans blue in the sample was determined by extrapolation from a standard curve generated using different concentrations of Evans blue in formamide. Results were expressed as Evans blue level/μg tissue.

2.6. Tissue sampling/preparation for subsequent analyses

Samples of the kidney and liver tissues collected at necropsy were weighed and homogenized (1/10 [*w*/*v*]) in physiological saline (0.9% [*w*/*v*] NaCl) using a Polytron homogenizer (T25 UtraTurax; IKA-Werke, Staufen, Germany). The homogenates were then centrifuged at 2486 × *g* for 20 min and the resultant supernatant collected and stored at −20 °C until used for biochemical assays. The pellets that remained were then re-suspended (1:8 [*w*/*v*]) in normal saline solution and then underwent three cycles of freeze-thawing (15 min each) within a −20 °C freezer. The materials were then centrifuged at ≈2500 × *g* for 20 min and the supernatant was collected and placed at −20 °C until used in measures of myeloperoxidase activity.

2.7. Evaluation of inflammatory cell infiltration

Myeloperoxidase (MPO) and eosinophil peroxidase (EPO) activities were measured as markers of sequestration/accumulation of, respectively, neutrophils and eosinophils in the studied tissues. Estimation of the activity of tissue MPO was performed according to the protocol of Krawisz et al. (1984). Results were calculated using a molar extinction coefficient (ε) value of 11.3 $M^{-1}\cdot cm^{-1}$ and are expressed in terms of mM H_2O_2 transformed/min/100 mg tissue (based on value of sample that underwent homogenization; see above). EPO activity was assessed based on the method of van Oosterhout et al. (1996). Results were expressed as change in absorbance/100 mg tissue.

2.8. Determination of tissue nitrite content and lipid peroxidation levels

The hepatic and renal homogenates were also measured for nitrite/nitrate (nitric oxide; NO) content using Griess reagent according to Sun et al. (2003). Results were expressed in μM NO/g tissue. Levels of lipid peroxidation were determined by the reaction with thiobarbituric acid (TBA), according to the method of Ohkawa et al. (1979). Results were calculated using a molar extinction coefficient of 1.56 × 105 $M^{-1}\cdot cm^{-1}$ and are expressed as nM MDA/per 100 mg tissue.

2.9. Estimation of glutathione level and catalase activity

The status of the anti-oxidant defense system in the tissues was assessed in the homogenates through estimates of levels of anti-oxidant catalase activity, according to protocol of Aebi, 1984 and of