Experimental and Toxicologic Pathology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Complement system and immunological mediators: Their involvements in the induced inflammatory process by *Androctonus australis* hector venom and its toxic components

Nadjia Bekkari [a], Marie-France Martin-Eauclaire [b], Fatima Laraba-Djebari [a,*]

[a] *USTHB, Faculty of Biological Sciences, Laboratory of Cellular and Molecular Biology, BP 32 El Alia, Bab Ezzouar, Algiers, Algeria*
[b] *Aix-Marseille University, CNRS UMR 7286 CRN2M, IFR Jean-Roche, Faculté de Médecine Nord, Bd Pierre Dramard, 13916 Marseille, Cedex 20, France*

ARTICLE INFO

Article history:
Received 25 November 2014
Received in revised form 12 March 2015
Accepted 2 April 2015

Keywords:
Aah venom
Ftox G50
AahI
AahII
Complement system
Cytotoxicity

ABSTRACT

Androctonus australis hector scorpion venom is well known by its high toxicity, it induces massive release of neurotransmitters that lead to pathophysiological disorders in cardiovascular, neuro-hormonal and immune systems. Previous studies have shown the relationship between the severity of scorpion envenoming and immune system activation. This study was assessed to investigate the involvement of complement system and inflammatory mediators after sublethal injection of *Aah* venom, its toxic fraction (FtoxG50) and its main toxins (AahI and AahII) into NMRI mice. The Activation complement system by the venom is also compared to that induced of lipoplysaccharides (LPS). Obtained results showed that seric complement system (CS) is activated by the venom and by its toxic components; this activation is more pronounced into liver tissue when toxic components (FtoxG50, AahI or AahII) are used. Increase of cytokine levels (IL1β, TNFα and ICAM) into hepatic tissue induced by AahI or AahII neurotoxins is correlated with tissue alterations. Aprotinin, a non specific inhibitor of complement system seems to be able to reduce CS consumption and to restore partially the induced tissue damage by venom. The mechanisms by which toxic fraction or LPS induced the activation of complement system seem to be different. Sensitivity of hepatic tissue is more pronounced after FtoxG50 injection; however lung tissue is more sensible to LPS than FoxG50.

1. Introduction

Inflammation is considered as a crucial event during pathogenesis induced by scorpion envenoming, it can be compared to that induced in sepsis. It involved several mediators and cellular actors which induce organ injuries. Correlation between envenoming severity and inflammatory intensity has been reported and characterized by an increase of inflammatory mediators as well as prostaglandins, cytokines and nitric oxide after scorpion sting or injection (De-Matos et al., 2001; Fukuhara et al., 2003; Adi-Bessalem et al., 2008)

Cytokines play crucial role in the evolution of inflammatory response; most of them are involved in the immune reactivity, tissue injury or repair and organ dysfunctions (Bhandari and Elias, 2006; Anderson et al., 2008; Wang et al., 2015).

It has been reported that *Androctonus australis hector* venom can increase the pro-inflammatory (IL1-β, IL-6, TNF-α) and anti-inflammatory cytokine levels accompanied by an activation of the complement system. It seems that these effects are correlated with tissue damage (Adi-Bessalem et al., 2008).

Complement system (CS) is considered as central effector of innate immunity. It acts by three pathways, the classical, the alternative and the lectin pathways. Activation of these pathways through proteolytic cleavage of precursor molecules lead to the release of anaphylatoxins C3a, C4a and C5a, and generation of membrane attack complex (Mocco et al., 2006). These anaphylatoxins are responsible of biological activities such as chemotaxy of leukocytes, degranulation of phagocytes, smooth muscle contraction and

Abbreviations: Aah, *Androctonus australis* hector; CS, complement system C3, C3a, C4a and C5a, complement components; CR1, complement receptors; CD55, cluster of differentiation; GPI, glyco-phosphatidyl inositol; IL1β, cytokines: interleukin 1β; TNFα, tumor necrosis factor α; ICAM, inter-cellular adhesion molecule; FtoxG50, toxic fraction of Aah venom; GVB-EDTA, gelatin veronal buffered-EDTA; GVB^{++}, gelatin veronal supplemented with $CaCl_2$ and $MgCl_2$; LPS, lipopolysaccharide; LD_{50}, lethal doses; MCP, membrane cofactor protein; NMRI, Naval Medical Research Institute mice; AahI and AahII, neurotoxins purified from Aah venom; RBC, red blood cells; RBCs, sensitized RBC.

* Corresponding author. Tel.: +00213 661516276; fax: +00213 21336077.
E-mail addresses: flaraba@hotmail.com, flaraba@usthb.dz (F. Laraba-Djebari).

http://dx.doi.org/10.1016/j.etp.2015.04.002
0940-2993/

increase of vascular permeability (Mollnes and Kirschfink, 2006; Bonifati and Kishore, 2007).

The activation of CS contributes to the pathogenesis of several inflammatory diseases (Mollnes and Kirschfink, 2006). A high level of cleavage components of CS were reported in several pathologies such as rheumatoid arthritis, psoriatic arthritis, cerebral injury and infections (Makinde et al., 1989; Elvington et al., 2012; Phieler et al., 2013; Ballanti et al., 2011). The inhibition of CS could be usefulness as a protective against their deleterious effects The involvement of CS in tissue injury following stroke was reported suggesting it's mediation by C3a anaphylatoxin (Mocco et al., 2006). Inhibition of CS leading to a reduction of tissue injury after cerebral ischemia has been also demonstrated (Arumugam et al., 2009).

To more understand the mechanism by which the inflammatory progression is developed through pathogenesis after scorpion envenoming, this study was undertaken to investigate the role of Complement System in the pathophysiological effects induced by Aah venom or its toxic components, compared to that induced by the LPS.

2. Materials and methods

2.1. Chemical reagent

The chemicals and reagents were of analytical grade and were purchased from Sigma (St. Louis, USA) or Merck (Darmstadt, FRG).

2.2. Biological materiel

1- *Animals:* NMRI mice (20 ± 2 g) were provided from Faculty of Biological Sciences of USTHB breeding, they are housed in a chamber with controlled temperature and humidity; throughout the experiment with free access to standard rodent diet and tap water. Experiments were carried out according to the European Community rules of the ethical Committee for animals' welfare.
2- Venom: *A. australis hector* (Aah) venom (provided from Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences (USTHB); it is lyophilized and stored at 4 °C). The lethal doses (LD_{50}) of *Aah* venom and FtoxG50) are estimated, respectively, to 0.85 and 0.50 mg/kg when injected by the i.p. route (Laraba-Djebari and Hammoudi, 1998)
3- FtoxG50: the major toxic fraction of *Aah* venom (Ftox G50: fraction obtained by fractionation of the venom by gel filtration on Sephadex G75.
4- Neurotoxins: Aah neurotoxins (AahI and AahII) are courtesy provided by Dr. Marie France Martin Eauclaire (UMR3132, CRN2 M, IFR jean Roche, University of the Mediterranean, Faculty of Medicine). The LD_{50} of Aah toxins AahI and AahII were estimated, respectively, to be 19 and 10 μg/kg (Zlotkin et al., 1971).
5- *Lipopolysaccharides* of *Escherichia coli* (Rough strains EH100 Ra mutant) and Aprotinin from bovine lung were purchased from Sigma Aldrich firm.
6- Cytokine kits of IL-1β, TNFα, and sICAM of mice were purchased from Amersham firm. Hemolysin is purchased from Biomerieux firm.

3. Methods

3.1. Animal experimentations

NMRI mice (20 ± 2 g) are divided into 6 groups of 36 mice. These subgroups were injected intraperitoneally and sacrificed according to a kinetic of 0.5, 1, 3, 6, and 24 and 48 h, blood, liver and lung are collected.

The first subgroup of mice was injected with *Aah* venom (0.50 mg/kg); the second one was injected with the toxic fraction (FtoxG50; 0.40 mg/kg). The fourth subgroup of mice was injected with a sublethal dose of neurotoxin AahI (10 μg/kg) and the fifth subgroup of mice was injected with a sublethal dose of neurotoxin AahII (8 μg/kg). The sixth subgroup of mice was injected with LPS of *E. coli* (0.40 mg/kg). Animals of group control were injected with physiological saline. In the pretreated group of animals, aprotinin was administrated 15 min prior to the venom, animals are then sacrificed (through external iliac vein), blood, liver and lungs, were collected at 3 and 24 h.

3.2. Hemolytic complement evaluation

Blood was centrifuged at 1460 × *g* and complement activity was evaluated in sera. Blood of sheep taken by jugular puncture into Alsever solution was stored at 4 °C for 10 days. Red blood cells (RBC) were washed three times by the GVB-EDTA, and their number is adjusted to 10^9 cells per ml of barbital buffer; RBC was sensitized in incubation with hemolysin. Sensitized RBC (RBCs) is stored at 4 °C up to 3 days. Samples are diluted in the 1/30 in GVB^{++} and incubated with RBCs for 30 min at 37 °C, then centrifuged at 1460 × *g* for 5 min. Hemolyzate was evaluated at 541 nm for sera and at 412 nm for tissue homogenates. Hemolysis (100%) was prepared by incubating RBCs with distilled water; free RBCs correspond to hemolysis (0%).

3.3. Myeloperoxidase activity (MOP)

Myeloperoxidase activity (MOP), marker of PMN sequestration in tissue, was determined using H_2O_2 as substrate and O-Dianizidine as chromogene (Bertazzi et al., 2003). Tissues (lungs and liver) were homogenized in Tris HCl buffer pH 6.6 with$Triton\text{-}X_{100}$ (1%) and centrifuged at 9167 × *g*. Supernatant (S1) was collected, and pellets are submitted to series of freeze thawing and re-suspended in Tris HCl (0.05 M pH 6.6) with Triton-X100, and centrifuged at 9167 × *g*. Absorbance was read at 460 nm after the addition of 100 μl of supernatant S1 to 100 μl of supernatant S2 and to 300 μl of chromogenic substrate (*O*-Dianizidine 0.167 mM in Tris–HCl buffer 0.05 M, pH 6.60.4 mM H_2O_2). MPO activity is evaluated using the difference between absorbance's each minute. Results are expressed by OD units/1 g of tissue.

3.4. Cytokines and intercellular adhesion molecule level

Cytokines (IL1β and TNFα) and ICAM-1 were assayed in liver tissue homogenates of groups of mice that receive AahI and Aah II toxins, by a specific two-site sandwich ELISA using Amersham Bioscience Kit (USA).

3.5. Histological analysis

Liver and lung are removed after 24 h of sample injection; they were fixed in phosphate-buffered formalin (10%) and embedded in paraffin, sliced (3 μm) and stained with hematoxylin and eosin for microscopic examination (Motic Digital Microscope PAL System).

3.6. Statistical analysis

All results were expressed as the mean ± SD. The statistical significance of differences between groups was analyzed by a Student *t*-test. Data were considered statistically significant if *p*-values were <0.01.

Please cite this article in press as: Bekkari N, et al. Complement system and immunological mediators: Their involvements in the induced inflammatory process by Androctonus australis hector venom and its toxic components. Exp Toxicol Pathol (2015), http://dx.doi.org/10.1016/j.etp.2015.04.002