Biologicals 44 (2016) 1–11

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Pathophysiological effects of *Cerastes cerastes* and *Vipera lebetina* venoms: Immunoneutralization using anti-native and anti-^{60}Co irradiated venoms

Sabrina Boumaiza, Habiba Oussedik-Oumehdi, Fatima Laraba-Djebari*

USTHB, Faculty of Biological Sciences, Laboratory of Cellular and Molecular Biology, BP 32, El-Alia, Bab Ezzouar, 16111, Algiers, Algeria

ARTICLE INFO

Article history:
Received 23 March 2015
Accepted 9 November 2015
Available online 8 December 2015

Keywords:
Viper
Venom
Pathophysiology
Toxicity
Gamma irradiation
Immunotherapy

ABSTRACT

Cerastes cerastes and *Vipera lebetina* are the most medically important vipers in Algeria. Their bite induces several pathological effects on victims of accidental envenomation. In this study we analyzed the pathogenesis induced after an experimental envenomation. Indeed, we determined, *in vitro*, venom enzymatic activities and we analyzed, *in vivo*, pathological effects induced on liver, heart, lung and skin. In addition we investigated the neutralizing potency of four experimental antivenoms elicited against native and irradiated venoms. Results revealed that *V. lebetina* and *Cerastes cerastes* venoms presented strong hemorrhagic, oedematic and necrotic activities. Histopathological study showed that both venoms induced deep damage in tissue structures leading to organ dysfunction. They also increased cellular peroxidases activities, indicating an inflammatory process that is known to amplify tissue damage. Western-blot analysis evidenced that anti-irradiated venoms recognized most components of native venoms. Antivenoms were effective in neutralizing all tested activities, with an increased protective effect obtained with anti-irradiated venoms. Anti-irradiated venoms reduced cellular peroxidases activities indicating a reduction of the inflammatory response. These results may improve our understanding of Algerian Viperidae bite pathogenesis and would encourage further studies planning to provide more proofs on the effectiveness of anti-irradiated venoms administration in the treatment of envenomation.

1. Introduction

Snakebite envenoming is a serious health problem in many countries especially in Africa, Latin America, and Asia [1]. Vipers are the main cause (more than 80%) of snake envenomations in all continents except in Australia where no specimens are recorded [2]. *Cerastes cerastes* (horn viper), widespread in North Africa, Egypt until Arabian Peninsula [3] and *Vipera lebetina* (Levantine viper) that is found in the South-East parts of Europe, the South-West of Asia and the North-West of Africa [4] are the most medically important Viperidae in Algeria.

Viper venoms are rich sources of pharmacological active peptides and proteins. These molecules interfere in several vital physiological functions, with high specificity and high potency. These molecules have local deleterious effects on the bite site and systemic effects resulting in coagulopathy, cardiopathy, neuropathy, oedema and necrosis [5–7]. It has been reported that snake venom serine proteinases (SVSPs) are mostly ascribed in the induction of systemic hemodynamic disturbances [3,8,9], phospholipases A2 (PLA2s) induce local myonecrosis and lymphatic vessel damage, whereas snake venom metaloproteinases (SVMPs) which play a relevant role in the pathogenesis of venom-induced local tissue damage [10–12] are responsible of local hemorrhage, extracellular matrix degradation, blistering and skin necrosis [13–15]. In addition, hyaluronidases and other proteases which have been detected in the venom of many snakes are spreading factors that act by degrading hyaluronic acid and other extracellular matrix constituents, and then facilitate the diffusion of other venom toxins [16,17]. Furthermore, many studies have demonstrated the increment of inflammatory cytokines in response to snake venoms [18,19].

* Corresponding author.
E-mail addresses: flaraba@hotmail.com, flaraba@usthb.dz (F. Laraba-Djebari).

http://dx.doi.org/10.1016/j.biologicals.2015.11.002
1045-1056/

Abbreviations

Anti iCc	anti-irradiated *C. cerastes* venom
Anti iVl	anti-irradiated *V. lebetina* venom
Anti nCc	anti-native *C. cerastes* venom
Anti nVl	anti-native *V. lebetina* venom
BSA	bovine serum albumine
Cc	cerastes cerastes
Co	cobalt
EPO	eosinophile peroxidase
Gy	gray
kDa	Kilo Dalton
MHD	minimum hemorrhagic dose
MOD	minimum oedematic dose
MPO	myeloperoxidase
NC	nitrocellulose
NMRI	Naval Medical Research Institute
OPD	*o*-Phenylenediamine
PBS	phosphate buffer saline
PLA2s	phospholipases A2
PMNs	polymorphonuclear neutrophils
SVMPs	snake venom metaloproteinases
SVSPs	snake venom serine proteinases

According to the World Health Organization [20], antivenom administration is the only specific treatment for snake envenomations. Antivenoms are formulations of immunoglobulins or immunoglobulin fragments, purified from plasma of hyperimmunized animals with venoms [21]. However, hyper immunization of antiserum-producer animals with native venom may induce severe pathological effects [11,22]. Gamma irradiation of toxic molecules can reduce their toxicity and other hydrolytic activities while maintaining enough structure to immunologically mimic the native venom [23–27,7]. Previous studies showed that treatment with ^{60}Co gamma radiation at a dose rate of 4.25 Gy/min with 2 kGy gamma rays is a suitable way to detoxify *C. cerastes* [7,26] and *V. lebetina* [27] venoms without affecting their immunogenicity. Gamma irradiation of snake venoms induces structural modifications of their proteins leading to the reduction or also the loss of their biochemical and biological activities that correlates with the reduction of their toxic potency [7,26,28,29].

The objectives of this work were to investigate the pathogenesis induced by experimental injections of the venoms of two endemic vipers of Algeria and to determine the neutralizing potency of four experimental antivenoms elicited against native and irradiated venoms.

2. Materials and methods

2.1. Animals

NMRI mice (20 ± 2 g body mass) were obtained from the animal breeding of the University of Sciences and Technology Houari Boumédiène (USTHB), Algiers. They were housed in temperature controlled rooms and received water and food *ad libitum* until used. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals.

2.2. Venoms and antivenoms

Crude venom of *Cerastes cerastes* (Cc) and *V. lebetina* (Vl) (supplied in lyophilized form) were obtained from Pasteur Institute of Algeria. Before use, they were dissolved in 0.15 M NaCl at a concentration of 20 mg/ml. Rabbit immune sera elicited against native *C. cerastes* or *V. lebetina* venoms (anti nCc or anti nVl) and irradiated *C. cerastes* or *V. lebetina* venoms submitted to ^{60}Co gamma radiation at a dose rate of 4.25 Gy/min with 2 kGy (anti iCc or anti iVl) were obtained from the Laboratory of Cellular and Molecular Biology, USTHB. Antivenoms elicited against *C. cerastes* or *V. lebetina* venoms were obtained using the same experimental protocol reported by Oussedik-Oumehdi and Laraba-Djebari [7] and Bennacef-Heffar and Laraba-Djebari [27] respectively.

2.3. Immune sera titration

Immune sera titration was assayed by ELISA test according to the method of Theakston et al. [30]. Microtiter wells of polypropylene 96-wells plates were coated with *C. cerastes* or *V. lebetina* venom (5 μg/ml) dissolved in Carbonate bicarbonate buffer 0.1 M, pH 9.6 for 1 h at 37 °C and overnight at 4 °C. Plates were blocked with 3% BSA diluted in PBS 0.1 M and serial dilutions of sera produced against native or irradiatd *C. cerastes* or *V. lebetina* venoms were added. After 1 h of incubation at room temperature, plates were washed with PBS/0.05% Tween$_{20}$. Antigen–antibody reaction was revealed with peroxidase-labeled anti-rabbit IgG conjugate (Sigma A-6154), diluted 1:2000 in PBS buffer – 5% skimmed milk, and followed by the substrate (10 mg of OPD in 10 ml of phosphate buffer and 10 μl of 30% H_2O_2). The reaction was stopped by the addition of 50 μl per well of 2 N H_2SO_4. The absorbance was read at 490 nm using ELISA microplate reader (Sanofi-Pasteur). The antibody titer was estimated as the inverse of the highest dilution that had an optic density greater than 0.05 at 490 nm.

2.4. SDS PAGE and western blotting

Venoms were submitted to SDS-PAGE according to Laemmli method [31] on Fisher Scientific FCVS10CBS system using acrylamide (12%). Briefly, venom samples (20 μg) diluted in Tris–HCl 0.0625 M, pH6.8 containing SDS (2%), glycerol (10%) and bromophenol blue (0.001%) in non-reducing conditions, were boiled for 5 min and applied to polyacrylamide gel. After migration, the gel was stained for 2 h with Coomassie brilliant blue R-250 solution (0.1%) made up freshly in acetic acid (10%). The gel was diffusion-destained by repeated washing in acetic acid (10%). Standard proteins used for calibration were α-Lactalbumin 14.4 kDa, Soybean trypsin inhibitor 20.1 kDa, Carbonic anhydrase 30 kDa, Ovalbumin 43 kDa, Bovine serum albumin 67 kDa and Phosphorylase b 94 kDa (PharmaciaBiotech, USA).

Western blot analysis was performed according to the method described by Towbin et al. [32]. Venoms submitted to 12% SDS-PAGE in non-reducing conditions were transferred onto a 0.45 μm Nitrocellulose (NC) paper on Fisher Scientific FCVS10CBS system according to the manufacturer's indications. NC paper was cut into strips and incubated, after blocking, with immune sera produced against native or irradiated venoms diluted 1:100 in 5% skimmed milk in PBS for 1 h at room temperature. After a washing cycle in PBS-0.1% Tween 20, strips were incubated with peroxidase-labeled anti-rabbit IgG conjugate (Sigma A-6154) for 1 h at room temperature. A last washing cycle was done prior to incubating the blots with peroxydase substrate containing α-Chloronaphtol, in the