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Combination of two antibody fragments F(ab′)$_2$/Fab: An alternative for scorpion envenoming treatment

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Abstract

Immunotherapy is the only effective treatment for scorpion stings. However the efficiency of this treatment varies depending on the forms of the antibodies and route of administration used. The antibodies are mostly injected as F(ab´)$_2$ fragments.
In this study, we investigated damage to the heart and lung tissue and the inflammatory response caused by *Androctonus australis hector* venom, its toxic fraction after molecular filtration or the isolated main alpha toxin (Aah II) in the presence or absence of different antibody molecules.
A mixture of antibody fragments, F(ab´)$_2$ and Fab, significantly reduced local leukocytosis, hemorrhage and inflammatory oedema induced by the *A. australis hector* venom and its toxins.

1. Introduction

Scorpion envenomation causes serious health problems in many countries. Clinical reports of victims stung by scorpions describe cardiac dysfunction and respiratory failure that can be fatal. Pulmonary oedema is a frequent complication in humans, especially in children [1–3]. The mechanisms underlying pulmonary oedema, respiratory failure and heart failure in these patients need to be fully elucidated [4,5]. Pulmonary oedema has been attributed to acute left ventricular failure resulting from massive catecholamine release or myocardial damage induced by the venom [6,7]. Pulmonary oedema may also result from increased pulmonary vascular permeability caused by vasoactive substances released by the venom. The activation of the inflammatory signaling cascade and release of lipid-derived mediators of inflammation may also be involved in the development of cardiorespiratory disorders [8–11].

Previous studies have examined the histopathological effects of scorpion venom in experimental models and in humans. Pulmonary oedema, disruption of myocardial fibers

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and alveolar and myocardial hemorrhages are frequently described in such studies [12–19]. One study demonstrated an influx of leukocytes into the bronchi and alveoli in rams envenomed with *Tityus discrepans* [20] and, in another study, leukocytosis was observed in the peritoneal cavity and peripheral blood of mice administered with *Tityus serrulatus* venom [21].

Most lethal accidents caused by scorpions in Algeria results from envenomation by the highly dangerous *Androctonus australis hector* (Aah). The venom of Aah contains three main alpha toxins (Aah I, Aah II and Aah III) that act at the voltage-gated sodium (Na_v) channels of excitable cells, such as nerve and muscle cells. These toxins are small molecular weight (7 kDa) basic peptides extensively cross-linked by four disulfide bridges. They appear to be responsible for nearly all lethal cases in mammals. They bind a specific site (site 3) on the Na_v channel, prolonging the inactivation phase of the channel [22].

The alpha toxin Aah II is the most abundant toxin in the venom and confers the highest toxicity ever described for a scorpion toxin either by subcutaneous injection (s.c) or intracerebroventricular (i.c.v) injection in mice. This toxin has a high affinity and high specificity for site 3 on both $Na_v1.4$ (Na_v channel from muscle cells) and Na_v 1.2 (Na_v channel found in brain) [23].

The severity of scorpion envenoming and the rapid diffusion of its toxins require specific treatment with immunotherapy. The administration of polyclonal specific antivenom remains the only treatment for severe scorpion envenomation; several studies highlight the beneficial therapeutic effects of antivenom [24–30]. These studies have also shown that other factors (the antibody form, the route and the time delay before their administration) can limit the clinical efficacy of such treatment.

In this study, we tested the effects of different antibody forms (Fab, F(ab´)$_2$, fragments or their mixture). We analyzed their efficiency by evaluating histopathological changes and inflammatory responses induced by Aah venom, its whole toxic fraction and its purified toxin Aah II in rats. Comparison of the effects of these different antibody forms provided further insight into treatment efficiency.

2. Materials and methods

2.1. Venom

Lyophilized crude Aah venom was supplied by the Research and Development Laboratory on Venoms at the Pasteur Institute of Algeria, collected from animals in the same area of the country. It was lyophilized and stored at 4 °C.

2.2. Animals

Male Wistar rats (200 ± 20 g body weight) were obtained from Pasteur Institute of Algeria. Rats were housed in temperature controlled rooms and received water and food *ad libitum* before being used for study. The experimental protocol was carried out according to the European Community rules of the Ethical Committee for Animals Welfare.

2.3. Reagents

All reagents were of analytical grade and were purchased from Sigma (St Louis, Missouri, USA), Prolabo (Darmstadt, Germany) and Merck (Darmstadt F.R.G).

2.4. Preparation of venom and toxic or non-toxic fractions

Aah venom toxic fraction F2 (FtoxG-50) and non-toxic fraction (F1) were isolated from the venom by gel filtration through Sephadex G50. Homogeneity was tested by SDS-PAGE electrophoresis and lethal potency was determined by the method of Behrens and Karber [31], previously described by Laraba-Djebari and Hammoudi [32]. The reference alpha toxin Aah II was prepared in the laboratory as previously described [22].

2.5. Purification of F(ab´)$_2$ and Fab anti-FtoxG-50 antibodies

Antibodies directed against the FtoxG-50 fraction were obtained from hyperimmunized horse sera. F(ab´)$_2$ and Fab fragments were prepared by hydrolysis with pepsin or papain respectively and purified on CNBr-activated Sepharose 4B. Their homogeneity and immunoreactivity were analyzed by SDS-PAGE and ELISA respectively. The neutralizing potency of these antibodies was determined in mice as previously described [33].

2.6. Experimental envenomation before and after treatment

All experiments were performed in groups of four rats. Intraperitoneal injections of Aah venom or its components and different antibody fragments, were administered for each rat (400 µl volumes). Concentrations of each solution injected were: 0.5 mg/kg body weight for whole venom, 0.4 mg/kg body weight for toxic and non-toxic fractions and 0.005 mg/kg body weight for Aah II toxin. Control groups were injected with 400 µl of 0.9% NaCl; antibody treatments (Fab, F(ab´)$_2$ and F(ab´)$_2$/Fab mixture 1:1) were used at 40 mg/kg body weight, 30 min after envenomation by intraperitoneal injection.

All animals were killed 24 h after injection of toxic compound and administration of treatment.

2.7. Histological analysis

Heart and lung extracts from animals injected only with Aah venom compounds or from envenomed animals further treated with different antibodies (described above) were immersed in formol fixative solution (10%) for 48 h at room temperature. They were embedded in paraffin, sliced (7 µm) and stained with Toluidin blue-Eosin for microscopic examination (Motic Digital Microscope PAL System).

2.8. Leukocyte recruitment

Animals were killed and dissected. Peritoneal cavities were washed several times in saline solution. Washing solution was recovered and analyzed to determine the different cell populations. Total cell counts were carried out using a hemocytometer (ADVIA, Hematology system). Leukocyte populations were identified and counted using Giemsa staining. The results are expressed as the mean ± SEM per ml of cell suspension.

2.9. Interpretation of the results

Results are expressed as means of four replicate measurements (means ± SD). $p<0.05$ was considered significance, using control values as a reference.