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Effect of cytokine antibodies in the immunomodulation of inflammatory response and metabolic disorders induced by scorpion venom

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ABSTRACT

Androctonus australis hector (*Aah*) venom and its neurotoxins may affect the neuro-endocrine immunological axis due to their binding to ionic channels of axonal membranes. This binding leads to the release of neurotransmitters and immunological mediators accompanied by pathophysiological effects. Although the hyperglycemia induced by scorpion venom is clearly established, the involved mediators in these deregulations are unknown. The strong relationship between inflammation and the wide variety of physiological processes can suggest that the activation of the inflammatory response and the massive release of IL-6 and TNF-α release induced by the venom may induce hyperglycemia and various biological disorders. We therefore investigated in this study the contribution of IL-6 and TNF-α in the modulation of inflammatory response and metabolic disorder induced by *Aah* venom. Obtained results revealed that *Aah* venom induced inflammatory response characterized by significant increase of inflammatory cells in sera and tissues homogenates accompanied by hyperglycemia and hyperinsulinemia, suggesting that the venom induced insulin resistance. It also induced severe alterations in hepatic parenchyma associated to metabolic disorders and imbalanced redox status. Cytokine antagonists injected 30 min prior to *Aah* venom allowed a significant reduction of inflammatory biomarker and plasma glucose levels, they also prevented metabolic disorders, oxidative stress and hepatic tissue damage induced by *Aah* venom. In conclusion, IL-6 and TNF-α appear to play a crucial role in the inflammatory response, hyperglycemia and associated complications to glucose metabolism disorders (carbohydrate and fat metabolism disorders, oxidative stress and hepatic damage) observed following scorpion envenoming.

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1. Introduction

Androctonus australis hector (*Aah*) venom is a complex mixture of mucopolysaccharides, amino acids, lipids, hyaluronidase, phospholipase, bioamines and neurotoxins, with various biological activities [1]. Neurotoxins, the main lethal components are responsible for almost biological effects induced by *Aah* venom. Many of these molecules are used as pharmacological tools in the understanding of signaling pathways, development of therapeutic agents and diagnosis [2].

Aah venom induced series of events, including stimulation of the neuro-endocrine immunological axis leading to the release of catecholamines, acetylcholine and immunological mediators such as pro-inflammatory cytokines [3,4]. These effects result mainly from sympathetic and parasympathetic stimulation of the autonomic nervous system by α-neurotoxins. These toxins are voltage-gated Na^+ channels (Nav) modulators; they block the fast-inactivation of the channel and induce a strong Na^+ entry in excitable cells [5]. Envenomed victims present multi-system-organ failure characterized by changes in hormonal biomarkers and an increase of blood glucose level. Although the hyperglycemia induced by scorpion venom is established, there are only few studies on the involved mediators in these deregulations [4,6].

It is well known that the high serum glucose levels are caused by the non-secretion of insulin or insulin resistance affecting consequently the glucose metabolism [7]. These disorders can induce metabolic deregulations, hepatic injury and oxidative stress, which, in turn deregulates the cellular functions leading to an over-production of reactive oxygen species (ROS) and/or to a reduction of antioxidant defense system [8]. Liver plays a central and crucial role in the regulation of carbohydrate and lipid metabolism and it is the focal organ of oxidative and detoxifying processes, thus the free radical generations and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including hyperglycemia [9,10]. Thus observed hyperglycemia following scorpion envenoming could be an important factor in the development and the evolution of pathophysiological effects induced by venom.

Previous studies have demonstrated that the activation of systemic inflammatory response induced by *Aah* venom leads to a massive production of pro-inflammatory cytokines such as IL-6 and TNF-α [3–5]. These inflammatory mediators are expressed in response to toxins, they play an important role in the pathogenesis of envenomation and appear to have a deleterious effect on patients and on experimental

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animal exposed to the scorpion venom [4,5,11]. We herefore investigated in this study, the effects of *Aah* venom on inflammatory response and glucose homeostasis and their consequences on hepatic functions, metabolic processes and redox status. We also tested whether cytokine antagonists (anti-IL-6 and anti-TNF-α) injection prior to *Aah* venom could prevent the induced pathophysiological effects.

2. Materials and methods

2.1. Venom

Aah venom was obtained by electric stimulation and provided by the Pasteur Institute of Algeria. It was lyophilized and stored at 4 °C.

2.2. Animal experiments and housing conditions

NMRI mice (20 ± 2 g body weight) were obtained from the animal breeding facility of Faculty of Biological Sciences, USTHB. They were housed in controlled temperature and humidity rooms, and received food and water *ad libitum*, with a natural cycle of light and darkness. Animals were used according to the European Community rules of the Ethical Committee for Animal Welfare. The experiments were achieved in line with the current guidelines for the care of laboratory animals.

2.3. Reagents

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

2.4. Animals and experimental protocols

Animals of experiments were divided into five groups (20 mice per group). Group 1 served as control was injected with saline solution (0.9% NaCl by s.c. route); Group 2 received a sublethal dose of *Aah* venom (0.5 mg/kg body weight by s.c. route); Groups 3 and 4 correspond respectively to the pre-treated animals with anti-IL-6 antibodies (0.5 mg/kg body weight; Sigma, St Louis, Missouri, USA) or anti-TNF-α antibodies (0.5 mg/kg body weight; Sigma, St Louis, Missouri, USA) injected by intraperitoneal (i.p.) route, 30 min before the injection of *Aah* venom (0.5 mg/kg body weight by s.c. route). The last group corresponds to animals co-injected with anti-IL-6 antibodies (0.5 mg/kg body weight; Sigma, St Louis, Missouri, USA) and anti-TNF-α antibodies (0.5 mg/kg body weight; Sigma, St Louis, Missouri, USA) by intraperitoneal (i.p.) route, 30 min before the injection of *Aah* venom (0.5 mg/kg body weight by s.c. route).

Animals were humanely sacrificed at different times after envenomation. Blood was then collected and sera were obtained after centrifugation at 3000 *g* for 10 min and kept at 4 °C until use. Organs and tissues (liver, pancreas, adipose tissue and skeletal muscle) were homogenized in physiological saline solution. Homogenates were centrifuged at 4000 *g* for 20 min and supernatants were used as tissue extract. Biomarkers of inflammatory response, metabolic disorders, oxidative stress and tissue damage were evaluated at 24 h after envenomation, however insulin secretion was determined according to a kinetic at 30 min, 60 min, 180 min to 1440 min after venom administration.

2.5. Peripheral blood cell counts

Blood samples were collected in EDTA tubes 24 h after *Aah* venom injection. A hemocytometer (ADVIA, Hematology system) was used for total cell count.

2.6. Evaluation of inflammatory cell infiltration

It was reported that liver, adipose tissue and skeletal muscle play a crucial role in the regulation of peripheral glucose homeostasis [12,13]. We therefore evaluated neutrophil and eosinophil accumulation in sera, liver, pancreas, adipose tissue and skeletal muscle homogenates.

Neutrophil accumulation and activation were estimated by assaying myeloperoxidase (MPO) activity as previously described [14]. Samples were mixed with orthodianisidine (0.167 mg/ml) and H_2O_2 (0.4 mM) in phosphate buffer. Absorbance was measured at 460 nm.

The extent of eosinophil accumulation in the biological samples was measured by assaying eosinophil peroxidase activity (EPO) as previously described [15]. Samples were mixed with Tris–HCl buffer containing OPD (10 mM) and H_2O_2 (0.4 mM). Absorbance was measured at 490 nm using an ELISA reader after incubation for 1 h at room temperature in the dark.

2.7. Biochemical assays

2.7.1. Determination of insulin levels and amylase activity

Plasma insulin concentrations were assessed at 30, 60, 180 and 1440 min after *Aah* venom injection using radioimmunoassay kit (INSULIN-CT, MP Biomedicals, Diagnostics Division, USA) according to the manufacturer's instructions.

Serum amylase is a common test used as biochemical markers for the diagnosis of acute pancreatitis. Amylase activity was determined in the sera by the colorimetric method of Winn-Deen and collaborators [16].

2.7.2. Determination of plasma glucose and hepatic glycogen content

Blood glucose was performed with glucometer (Accu-Check, Roche, Dublin, Ireland), avoiding to stress the animal.

Hepatic glycogen content was evaluated in different groups using colorimetric method. It was determined by treatment with HCl (37%) and alcohol (95%) [17]. Hepatic glycogen extract was diluted in 2 ml of distilled water and a drop of Lugol. The developed brown color in the reaction was measured at 470 nm.

Glycogen was also assessed by histochemical method. Histological analysis was performed on extracted livers after their immersion in formalin fixative solution (10%) for 48 h at room temperature. They were embedded in paraffin, sliced (7 μm) and stained with the Periodic acid–Schiff (PAS) reaction and hematoxylin for microscopic examination (Motic Digital Microscope PAL System). The reaction is considered positive when the region is colored in purple or magenta [18].

2.7.3. Evaluation of lipid metabolites

2.7.3.1. Evaluation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Plasmatic transaminase (AST and ALT) activities are used as biochemical markers to evaluate hepatic damage [19]. The blood of mice was collected without anticoagulant, 24 h after envenomation. The serum were obtained by centrifugation of blood at 3000 *g* for 10 min and used to evaluate plasmatic transaminase activities. The activities of aspartate and alanine transaminases (AST and ALT) were assayed by enzymatic method [20] using a commercial kit (Spinreact, S.A. Spain) according to the manufacturer's instructions. The enzyme values were expressed in International Units (IU/l) and presented as the mean of 3 replicate determinations (means ± SD).

2.8. Histological analysis

Liver extracted from animals was immersed in formol fixative solution (10%) for 48 h. It was embedded in paraffin, sliced (7 μm) and stained with hematoxylin and eosin for microscopic examination (Motic Digital Microscope PAL System).

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