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Diagnosis of *Mesobuthus eupeus* envenomation by skin test: Reverse passive Arthus reaction

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ABSTRACT

While being stung by two large families of scorpions, Buthidae and Scorpionidae have different symptoms and complications, a similar maintenance treatment usually considers as the scorpion species could not be identified easily. Therefore, this study was an attempt to develop an immunologic response for designing a skin sensitivity test that can be used to determine the poisoning.

The sensitivity and the specificity of RPA reaction for detecting experimental envenomated mice were evaluated. The inflammatory response for detection of envenomation was obtained by the injection of a solution containing complement, polyelectrolytes and purified monovalent antibodies.

As the result, 84.44% sensitivity and 100% specificity recorded 15 min after challenge. Macroscopic findings were also confirmed histologically. No cross-reactions were observed with other species of scorpions and snake venoms. Designed Skin test induced obvious inflammatory reaction without any histological lesions. Besides adding the complement components and polyelectrolyte to the monovalent antibody leads to an increased susceptibility of inflammatory cells in this reaction, resulting in forming a visible inflammation in a short time. According to satisfactory specificity and sensitivity and visible results in about 15 min, non-harmful and cost benefity of reverse passive Arthus test can be used for diagnosis of scorpion envenomation.

1. Introduction

Scorpion sting is a health problem in many tropical and subtropical countries. It is the most common cause of human deaths among the venomous creatures. Reported deaths due to scorpion sting are about ten times higher than the snake bites. Some studies have noted that 32 species of the Iranian scorpion fauna belong to the two families of buthidae and scorpionidae out of which 7 could be dangerous to human. *Mesobuthus eupeus* which is found in South and Central parts of Asia belongs to the Buthidae and it is the most responsible for scorpion stings in mentioned regions (Karatas et al., 2012; Karatas, 2003; Sadeghian, 2003). Its venom contains several toxins with many of harmful and deadly effects on envenomated

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patients (Sagheb et al., 2012; Tuuri and Reynolds, 2011; Khoobdel et al., 2013) .

Two large families of scorpions, Buthidae and Scorpionidae have different envenomation symptoms and complications. Some of them lead to acute hemolytic anemia while the others have different neurological or cytotoxic disorders (Amitai, 1998; Jalali et al., 2010) .These various disorders need specific medical treatments in stung persons (Binder, 1989) but as the scorpion species cannot be identified, the same maintenance treatment considered unfortunately; this could endanger the patients' lives, prolong the therapeutic process or at least raise the costs .Therefore, a skin sensitivity test, which can be used to differentiate the various envenomation types within a relatively short time (15–20 min), is valuable.

Arthus first described local anaphylaxis in 1903. Intradermal injection of antigen to animals that have been previously sensitized could cause Arthus reaction at the injection site. Immune complex formation and deposition also cause acute inflammatory responses. Arthus reaction, an immunocomplex disease, is a model of type III hypersensitivity. It is necessary to remind that complement activation and degranulation of phagocytes, mast cells and basophils cause contraction of vascular smooth muscles and increase permeability of the capillary veins (Paul, 2008). In reverse Arthus, serum is injected into the skin while antigen injected intravenously. This reaction is passive, because the injected antibody attained from another animal. Reverse passive Arthus reaction has been used for pathological immune complexes determination. Response begins with intravenous injection of antigen and subsequently intradermal injection of antibody. Histological changes are characterized by leukocyte infiltration, edema, necrosis, and hemorrhage. Neutrophils recruitment and complement activation are necessary in this pathway; although the role of other cells in leukocyte stimulation and edema formation are not well defined yet. Mast cells are located near the blood vessels, and by stimulation, they can release their proinflammatory factors such as histamine, platelet-activating factor, leukotrienes, oxygenase products and cyclooxygenase. The released peptides of the reaction can attract multi-nuclear cells. Thereafter releasing lysozyme by the multi-nuclear cells leads to enhancement of blood vessels permeability. The mast cell activation process may also occur by complement-derived peptides (Zhang et al., 1991). Concentration and deposition are the effects of electrostatic charge on immune complex pathogenesis.

In this study, sensitivity and specificity of RPA reaction for diagnosis of experimental envenomated mice were evaluated by the injection of a solution containing complement, polyelectrolytes and purified monovalent antibodies attained from immune rabbits against *M. eupeus* venom.

2. Material and methods

2.1. Venom

The *M. eupeus* scorpions were collected from Khuzestan province with 31°19′–32°73′N, 48°41′–49°4′E in the South West of Iran and were milked by electric stimulation. The freeze-dried venom was dialyzed and centrifuged and the supernatant was collected. The protein content of venoms and the samples were determined by the absorbance at 280 NM with Bovine Serum Albumin (BSA) as standard. To determine the level of toxicity, LD50 was calculated using the Spearman–Kaerber method.

2.2. Antibody

Outbreed New Zealand white male rabbits were acclimated to room temperature for 2 weeks prior to immunization. The methods of immunization were the same as those described previously (Inceoglua et al., 2006). The immunized blood was directly collected into sterilized tubes and allowed to clot. Serum was pipette out, centrifuged at 1500 RPM for 10 min, isolated in a sterilized vial and finally stored in 4 °C for bioassay tests.

Polyclonal antibody was first purified by ammonium sulfate precipitation (50% saturation for the final solution), dialyzed in PBS, and then subjected to an affinity column conjugated with venom. The column was prepared by conjugating 20 mg of venom with 7 ml of activated CH-Sepharose 4B (Sigma–Aldrich, Product Number: 4 B200). Cyanogen bromide was activated by the method of Cuatrecasas (March et al., 1974). Antibody was eluted from the column with 0.1 M Glycine (pH 2.5) and fractions were collected and neutralized immediately by adding an appropriate amount of 1 M Tris-PH 9 to each fraction.

2.3. Production and purification of Fab2 fragments of specific antibody

To generate F(ab)2 fragments, the specific antibody was digested with pepsin (Sigma–Aldrich, Product Number: P6887). In summary, after purification of IgG antibody, the antibody was digested with pepsin in 0.1 M acetate buffer (pH 4.0). The reaction was quenched by adding a few drops of 1.0 M Tris base to pH 8.0. Purification of the Fab2 was done according to Silanes et al. (Silanes et al., 2009).

2.4. SDS-PAGE analysis

All samples were analyzed by Sodium Dodecyl sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). According to the Laemmli method (Laemmli, 1970), the concentration of acrylamide was taken as 11%.

2.5. Skin testing

24 h after shaving the abdominal area of the mice, 100 μg of the scorpion venom in the volume of 0.01 ml was injected subcutaneously while it is necessary to recall that the amount of venom that a sting may inoculate is about 0.1–0.6 mg. The accurate amount of the antibody for stimulation of the reactions was calculated by injecting 50–200 μg of specific antibodies in the volume of 0.02 ml at 2 cm away from the toxin injected area after 30 min subcutaneously. The site was checked grossly for redness and inflammation 5, 10, 15, 20, 30, 60, 120, 240, 480, 720, 1440 and 2880 min after the injection. For accelerating the reaction, 25–100 μg of complement sera from rabbit (Sigma,