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Review

Evolution of alternative methodologies of scorpion antivenoms production

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ABSTRACT

Scorpionism represents a serious public health problem resulting in the death of children and debilitated individuals. Scorpion sting treatment employs various strategies including the use of specific medicines such as antiserum, especially for patients with severe symptoms. In 1909 Charles Todd described the production of an antiserum against the venom of the scorpion *Buthus quinquestriatus*. Based on Todd's work, researchers worldwide began producing antiserum using the same approach *i.e.*, immunization of horses with crude venom as antigen. Despite achieving satisfactory results using this approach, researchers in this field have developed alternative approaches for the production of scorpion antivenom serum. In this review, we describe the work published by experts in toxinology to the development of scorpion venom antiserum. Methods and results describing the use of specific antigens, detoxified venom or toxins, purified toxins and or venom fractions, native toxoids, recombinant toxins, synthetic peptides, monoclonal and recombinant antibodies, and alternative animal models are presented.

1. Introduction

1.1. Biologic generalities

Scorpions inspire fear due to their venomous sting as well as to their fearful appearance. On the other hand, they also inspire fascination, due to their imposing behavior and predatory habits. They were venerated by ancient Egyptians and sacred to Isis, the goddess of rebirth. Having adapted to terrestrial ecosystems over the last 425 million years they represent one of the best-adapted terrestrial arthropods occupying most tropical and temperate zone habitats without alterations to their anatomy over time and between species (Beccaloni, 2009). Irrespective of genetic divergence, their morphology has not undergone great variation, basically corresponding to 3 ecomorphological patterns mostly relating to the substratum type (*i.e.*, lithophilic, psammophilic, and pelophilic scorpions) (Beccaloni, 2009), body size, and the combinational armor: large palps *vs.* reduced telson/weak venom and vice versa (Mebs, 2002; Prendini and Wheeler, 2005).

According to an updated and reliable internet site operated by Jan Ove Rein (http://www.ntnu.no/ub/scorpion-files/index.php), there are more than 2000 extant species comprising 195 genera and 15 families. Of these, only about 25–50 species (according to various resources e.g., http://scorpion.amnh.org and http://www.who.int/emc/amr.html; Chippaux and Goyffon, 2008) are considered to be of medical importance. With the exception of only one species which belongs to the Hemiscorpiidae family (*Hemiscorpius lepturus*) endemic to Iran, Iraq, Pakistan, and Yemen (Chippaux and Goyffon, 2008; http://www.ntnu.no/ub/scorpion-files/h_lepturus.htm), all other scorpion species posing serious danger to humans belong to the family Buthidae. Genera that draw the most attention in many countries and in relevant literature due to the morbidity

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associated with their venoms are: *Centruroides* (North and Central America, Colombia), *Tityus* (South America), Androctonus, Leiurus, Buthus (North Africa and Middle East), *Parabuthus* (South Africa), and *Mesobuthus* (India) (Chippaux and Goyffon, 2008; Beccaloni, 2009). However, not all species belonging to the above genera are dangerous to humans. For example, the venom of *Mesobuthus gibbosus* endemic to the Balkan Peninsula and Turkey is milder compared to that of *Mesobuthus tumulus* endemic to India (Beccaloni, 2009).

1.2. Scorpionism

Scorpionism is a neglected public health threat that refers to accidents associated with scorpion stings and is considered a serious health issue in developing countries (WHO, 2007). Reports describing accidents involving scorpion stings associated with greatest severity primarily originate from Central and South America, North Africa, the Middle East, and Southwest Asia (Chippaux and Goyffon, 2008).

The main symptoms associated with scorpion stings are intense local pain, sweating, tachypnea, tachycardia, agitation, pancreatitis, pulmonary edema, hemolysis, and necrosis (Espino-Solis et al., 2009; Borchani et al., 2011a, 2011b; Khattabi et al., 2011). The severity of scorpion envenomation depends on the body mass, sensitivity of the victims, the amount of venom injected, and the species of scorpion.

After snakebites, scorpion stings represent the primary source of human envenomation worldwide (Mebs, 2002; Espino-Solis et al., 2009). Scorpionism represents a serious public health problem mainly in North Africa, the Middle East, and Central and South America. However, in areas of the world where adequate care is available, the use of appropriate antivenoms and others medical care has resulted in impressive decreases in mortality rates (Chippaux et al., 2011; Chippaux and Goyffon, 2008).

The treatment of scorpionism aims to combat symptoms using analgesics and serotherapy. The effectiveness of the treatment is directly related to the amount of time that elapses between the accident and the administration of the antivenom. According to the Brazilian Ministry of Health, antivenom must only be administered intravenously by doctors in a hospital setting. The patient's vital signs must be constantly monitored, and any changes to the patient's clinical status requiring medical intervention must be rapidly performed by trained medical staff (Ministério da Saúde, 2001).

1.3. The epidemiology of scorpionism

1.3.1. Old world

Among North African countries, the highest rates of scorpionism originate in Tunisia (420 per 100,000 inhabitants) (Njah et al., 2001) resulting in 50 deaths/year (Mansour, 2001); although more recent reports indicate that Algeria may now have higher incidence rates (Chippaux et al., 2011). Incidents rates increase from north to south and between rural and urban communities (Chippaux and Goyffon, 2008). The most dangerous species in these parts of the world are *Androctonus australis*, *Androctonus mauretanicus*, *Androctonus aeneas*, *Leiurus quinquestriatus*, *Buthus occitanus*, and *Hottentota franzwerneri*. In sub-Saharan Africa, fewer cases of hospitalization and death have been reported (Bergman, 1997; Attamo et al., 2002) with most cases presenting during the summer in younger victims (Chippaux and Goyffon, 2008). Inadequate health systems, combined with the misuse or unavailability of antivenoms in these areas is associated with high mortality rates (Chippaux et al., 2011). A similar incidence pattern associated with a significant reduction in mortality rates was observed following recent improvements to the health care system in the Middle East, for example, Saudi Arabia (Al-Sadoon and Jarrar, 2003); however, high mortality rates still exist in rural settings (Mahaba, 1997). Due to sparse epidemiological data in India the number of scorpionism incidents are difficult to estimate; however, fatal accidents are regularly reported (unofficially) and are mostly due to *Mesobuthus tamulus* stings (Chippaux and Goyffon, 2008).

1.3.2. New world

In Latin America, incidence rates increase during summer months (Schenone and Fontecilla, 1998; De Roodt et al., 2003; Chowell et al., 2005) mostly in suburban areas (Celis et al., 2007; Schenone and Fontecilla, 1998), and involve primarily adults instead of children (Chowell et al., 2005; Celis et al., 2007). Due to the presence of many species adapted to urban habitats (*e.g., Tityus trivittatus*) many incidents occur in domiciles (De Roodt et al., 2003). In Mexico, incidence rates and scorpion sting severity is affected by location, that is, cases are more common in urban settings and in populations <2500 inhabitants (Celis et al., 2007) and they are more frequent in adults than in children (Chowell et al., 2005; Celis et al., 2007). Another factor affecting incidence rates is climate, with the majority of stings occurring at the end of the dry season (Chowell et al., 2005). Mortality in these areas has dramatically decreased over the last decades due to the availability of antivenom against *Centruroides* spp. (from 0.6 to 0.07 per 100,000 inhabitants) (Celis et al., 2007). Presently, mortality rates remain rather high in children younger than 5 years old and in elders older than 60 years old (Celis et al., 2007). In Mexico, species of the genus *Centruroides* are considered the most dangerous, while in the rest of Latin America species of the genus *Tityus* represent the highest risk for humans with many species inhabiting vast areas and many being endemic.

In most of the countries above fatal envenomations have dramatically decreased during the last decades due to widely used antivenoms available for the treatment of stings from different species. However, the rates of fatal envenomations vary significantly between regions, with some rural areas still reporting high mortality rates (*e.g*, 6803 stings and 6.6 deaths per 100,000 inhabitants annually in some rural places of the Andes) (De Sousa et al., 1996). *Tityus serrulatus* causes approximately 10,000 cases annually with 50% occurring in the state of Minas Gerais (Maria et al., 2005) where mortality reaches 1.1%, especially among children (Cardoso et al., 1995; Freire-Maia and Campos, 1989). *T. serrulatus* is well adapted to both rural and urban environments and is responsible for most envenomations reported in southeast and northeast Brazil where it is most common.

1.4. Scorpion venoms and their toxins

The toxic arsenal of scorpion venoms are composed mainly of neurotoxins that act on ion channels (*i.e*., sodium, potassium, calcium, and chloride channels). Toxins acting on sodium and potassium channels are considered the most toxic components of scorpion venom and show sequence similarity in their primary and tertiary structures. These toxins consist of one α-helix and 2-3 β-sheets stabilized by 3–4 disulfide bridges (Rodrígez de la Vega and Possani, 2005; Rodrígez de la Vega et al., 2010; Chugunov et al. 2013). However, some enzymes present in venom such as hyaluronidases, proteases, and phospholipases also contribute to symptoms associated with scorpion venom envenomation (Costal-Oliveira et al., 2012; Borchani et al., 2013; Venancio et al., 2013; Horta et al., 2014; Carmo et al., 2014).

Scorpion toxins acting on ion channels can be further classified based on the type of channels affected, pharmacological effects, and their primary sequences. Toxins acting on sodium channels are