Toxicon xxx (2013) 1–11

Contents lists available at SciVerse ScienceDirect

Toxicon

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

Enzymatic properties of venoms from Brazilian scorpions of *Tityus* genus and the neutralisation potential of therapeutical antivenoms

Emerson J. Venancio [a,b], Fernanda C.V. Portaro [b], Alexandre K. Kuniyoshi [b], Daniela Cajado Carvalho [b], Giselle Pidde-Queiroz [b], Denise V. Tambourgi [b,*]

[a] *Immunology Laboratory, State University of Londrina, Londrina, PR, Brazil*
[b] *Immunochemistry Laboratory, Butantan Institute, 05503-900 São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 6 November 2012
Received in revised form 23 January 2013
Accepted 19 February 2013
Available online xxxx

Keywords:
Scorpion venom
Tityus spp.
Metalloproteinases
Dynorphin 1-13
Leu-enkephalin
Antivenoms

ABSTRACT

Tityus scorpion stings are an important public health problem in Brazil, where the incidence of such stings exceeds the incidence of the health problems caused by other venomous animals, including snakes. In this study, we have analysed specific enzymatic activities of the venom from the Brazilian scorpions of *Tityus* genus, *i.e.*, *Tityus serrulatus*, *Tityus bahiensis* and *Tityus stigmurus*. The data presented here revealed that *Tityus* spp. venoms exhibited significant hyaluronidase activity but no phospholipase activity. All the venom samples exhibited the ability to hydrolyse Abz-FLRRV-EDDnp and dynorphin 1-13 substrates. These activities were inhibited by 1,10-phenanthroline but not by PMSF, indicating the presence of metalloproteinases in the *Tityus* spp. venoms. The venom peptidase activity on Abz-FLRRV-EDDnp and on dynorphin 1-13 was partially inhibited by therapeutic Brazilian anti-scorpion and anti-arachnidic antivenoms. Dynorphin 1-13 (YGGFLRRIRPKLK) contains two scissile bonds between the residues Leu-Arg and Arg-Arg that are susceptible to cleavage by the *Tityus* venom metallopeptidase(s). Their cleavage releases leu-enkephalin, an important bioactive peptide. The detection of metalloproteinase(s) with specificity for both dynorphin 1-13 degradation and leu-enkephalin releasing can be important for the mechanistic understanding of hypotension and bradycardia induction in cases of scorpion stings, whereas hyaluronidases might contribute to the diffusion of the toxins present in these venoms. Furthermore, the limited inhibition of the toxic enzymatic activities by commercial antivenoms illustrates the necessity of improvements in current antivenom preparation.

1. Introduction

Scorpionism is a major public health threat in Brazil, where scorpion-related accidents far outnumber those of other venomous animals, including snakes. Data provided by the Information System (SINAN, *Sistema Nacional de Informação de Agravos de Notificação*) of the Brazilian Ministry of Health show that from January 2007 to December 2011, there were 235,892 cases of scorpionism in Brazil and 414 deaths. The actual number of accidents is likely underestimated, as most of these accidents are not

Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl-phosphate; BSA, bovine serum albumin; CTAB, cetyltrimethylammonium bromide; EDTA, ethylene diamine tetracetic acid; ELISA, Enzyme-Linked Immunosorbent Assay; FRET, fluorescent resonance energy transfer substrate; IgG (GAH/HRP), goat anti-horse IgG labelled with horseradish peroxidase; IgG (GAH/AP), goat anti-horse IgG labelled with alkaline phosphatise; NBT, nitroblue tetrazolium; OPD, ortho-phenylenediamine; PMSF, phenylmethanesulfonyl fluoride.

* Corresponding author.
E-mail addresses: dvtambourgi@butantan.gov.br, dvtambourgi@yahoo.com (D.V. Tambourgi).

0041-0101/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxicon.2013.02.012

severe and do not require antivenom (Ministério da Saúde, 2001). In recent years, there has been an increase in the number of scorpions accidents that is likely attributed to the ability of certain species to adapt to densely populated areas, resulting in uncontrolled population growth within cities (Soares et al., 2002). Furthermore, the combination of high temperatures and humidity increases the incident rate during the summer months, when scorpions become more active (Barbosa et al., 2012). Currently, approximately 70% of scorpionism cases occur within urban areas, in or around residences. Scorpion accidents occur more in individuals between 20 and 49 years of age. However, the largest proportion of deaths is observed in individuals younger than 14 years of age (Ministério da Saúde, 2001).

Symptoms resulting from scorpion stings are variable and can be grouped into three stages depending on the severity of the poisoning. In most cases, the initial envenomation is benign and reaches stage I, which is characterised by intense pain in most cases (stage Ia), as well as stirring, fever, sweating, nausea and blood pressure fluctuation (Stage Ib). Severe cases progress from Stage I to Stage II (5–10% of cases), which is characterised by sweating, vomiting, cramps, diarrhoea, hypotension, bradycardia, pulmonary obstruction and dyspnoea. The last and most dangerous stage is Stage III, which is characterised by respiratory complications such as pulmonary oedema, bronchospasm, and cyanosis and can be associated with hyperthermia, cardiac arrhythmia and myocardial ischemia (Chippaux and Goyffon, 2008). The severity of scorpion envenomation is much greater in children but varies with the scorpion species, age, and size (Amitai, 1998). The treatment of scorpion accidents involve symptomatic measures, support of vital functions, and, in severe cases, serum therapy.

The genus *Tityus* contains the largest number of scorpion species. Over 60% of scorpions found in tropical and subtropical regions belong to this genus (Ministério da Saúde, 2001). In Brazil, the three *Tityus* species *Tityus serrulatus* (yellow scorpion), *Tityus bahiensis* (brown scorpion), and *Tityus stigmurus* are the main causes of scorpionism in humans (Bucaretchi et al., 1995; Eickstedt et al., 1996).

Tityus serrulatus is the Brazilian scorpion that causes the most serious accidents, with mortality rates of approximately 1% among children and the elderly. This species is widely distributed throughout the country, reaching the states of São Paulo, Minas Gerais, Bahia, Espírito Santo, Goiás, Paraná and Rio de Janeiro (Ministério da Saúde, 2001). One of the factors contributing to its proliferation and distribution is the ability to reproduce by parthenogenesis (Lourenço, 2008) which complicates the control of these arachnids.

T. stigmurus is another scorpion species of clinical relevance, which is also capable of parthenogenesis and is distributed predominantly in the northeastern region of the country. Studies conducted between 1990 and 1995 in the state of Bahia, Brazil, have shown that *T. stigmurus* as the main cause of scorpionism within the region (Lira-da-Silva et al., 2000). However, due to the lack of information, the actual epidemiological impact of these incidents of scorpionism within these localities remains relatively obscure.

Unlike the species mentioned above, *T. bahiensis* exhibits crossbreeding, which requires encounters between males and females during certain periods of the year. This scorpion can be found in the states of São Paulo, Minas Gerais, Goiás, Mato Grosso do Sul, Paraná, Rio Grande do Sul and Santa Catarina (Porto et al., 2010).

Scorpion venom is a complex mixture of components that can be separated into an insoluble, generally non-toxic, fraction and a soluble fraction containing toxic peptides that exhibit activity on ion channels, in addition to mucopolysaccharides, nucleotides, vasoactive amines (serotonin or histamine), protease inhibitors and enzymes (Gazarian et al., 2005; Rodríguez de la Vega et al., 2010). In general, it is believed that the toxic activity of scorpion venom is predominantly attributed to the presence of peptides that disrupt Na^+, K^+, Ca^+ and Cl^- channels in neuronal cells (Possani et al., 2000). Recent studies have shown that apart from these peptides, several molecules that play a role in scorpion poisoning or that exhibit properties of biotechnological interest are also present in scorpion venoms (Wu et al., 2010; Zeng et al., 2012; Zhao et al., 2011).

The specific treatment for *Tityus* envenomation is the intravenous administration of heterologous antivenoms. Recently, a double-blind study evaluating the effectiveness of the serum therapy in the treatment of children who were stung by scorpions and admitted to intensive care units showed that the specific $F(ab')_2$ antivenom is extremely effective in reversing the symptoms of poisoning, reducing the use of sedation and of circulating levels of venom (Boyer et al., 2009). In contrast, other studies have found no significant benefit in the administration of antivenom to patients stung by scorpions (Abroug et al., 1999). There is evidence that the antivenom is ineffective in the treatment of severe cardiovascular manifestations due to the involvement of the autonomic nervous system in the aetiology of these manifestations (Amaral and Rezende, 2000).

In Brazil, the following two therapeutic scorpion antivenoms are produced by the Butantan Institute: 1) an anti-arachnidic antivenom, which is obtained by the immunisation of horses with a mixture of venoms derived from *T. serrulatus* (57%), *Phoneutria nigriventer* (21.5%) and *Loxosceles gaucho* (21.5%), and 2) an anti-scorpionic antivenom, which is obtained by the immunisation of horses with a mixture of venoms derived from *T. serrulatus* (50%) and *T. bahiensis* (50%). Based on the potential diversity of composition and toxicity of *Tityus* spp. venoms, the therapeutic antivenoms might insufficiently recognise the major components of distinct venom species occurring throughout the country. Therefore, the aim of this study was to characterise the enzymatic properties of venoms derived from *T. serrulatus*, *T. bahiensis* and *T. stigmurus* and to evaluate their antigenic cross-reactivity using the Brazilian antivenoms, as well as to test the ability of these antivenoms to neutralise the enzymatic activities of these venoms.

2. Materials and methods

2.1. Chemicals and reagents

Triton X-100, Tween-20, bovine serum albumin (BSA), ethylene diamine tetracetic acid (EDTA), cetyltrimethylammonium bromide (CTAB), ortho-phenylenediamine (OPD), hyaluronic acid, 1,10-phenanthroline, phenylmethanesulfonyl

Please cite this article in press as: Venancio, E.J., et al., Enzymatic properties of venoms from Brazilian scorpions of *Tityus* genus and the neutralisation potential of therapeutical antivenoms, Toxicon (2013), http://dx.doi.org/10.1016/j.toxicon.2013.02.012