Toxicon 94 (2015) 45–49

Contents lists available at ScienceDirect

Toxicon

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

Effects of *Tityus serrulatus* scorpion venom on thromboelastogram in rats

Thiago A. Lisboa [a], Marcus Vinícius M. Andrade [b], João B. Rezende-Neto [c], Maria Júlia M. Silva [d], Mário G. Carvalho Jr. [a], Tasso Moraes-Santos [e], Daniel D. Ribeiro [f], José Renan Cunha-Melo [g, *]

[a] Federal University of Minas Gerais, Brazil
[b] Department of of Internal Medicine, Federal University of Minas Gerais, Brazil
[c] St. Michael's Hospital, University of Toronto, Canada
[d] Pontifical Catholic University of Minas Gerais, Brazil
[e] Department of Pharmacy, Federal University of Minas Gerais, Brazil
[f] Department of Hematology, Federal University of Minas Gerais, Brazil
[g] Department of Surgery, Federal University of Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 9 July 2014
Received in revised form
4 October 2014
Accepted 7 October 2014
Available online 7 November 2014

Keywords:
Scorpion venom
Hypercoagulability
Thromboelastometry
Rats

ABSTRACT

Thromboelastometry was used to evaluate blood coagulation in anesthetized rats after intravenous administration of *Tityus serrulatus* scorpion venom (Tx). Tracheostomy followed by catheterization of the left jugular vein and right carotid artery were performed for Tx or Ringer's lactate solution injection and blood sample harvesting, respectively. Blood samples were obtained at the beginning of the experiments (baseline) and at two, five, 15, 30, and 60 min after intoxication. The following coagulation parameters were analyzed: CT (Clotting Time), CFT (Clotting Formation Time), Alpha Angle (α), MCF (Maximum Clot Firmness) and TPI (Thrombodynamic Potential Index). Toxin-induced hypercoagulability was demonstrated at the 15 and 60 min. We hypothesize Tx-induced hypercoagulability and enhanced clot formation could be explained by catecholamine release, systemic inflammatory response, and complement system activation, at least in the first hour after envenomation. Further studies are needed to determine the molecular mechanism of Tx-induced coagulopathy.

1. Introduction

In recent years, the Brazilian Ministry of Health reported an increase in accidents caused by scorpion stings, which is considered a public health problem (SINAN/SVS/MS, 2012). Most cases occur in the state of Minas Gerais and particularly in its capital city of Belo Horizonte during the summer. *Tityus serrulatus* species accounts for fatal stings, especially in children and elderly people (Clot-Faybesse et al., 2000). Previous studies have shown that severe cases that lead to death are mostly children and related to delayed first medical care (Guerra et al., 2008). Victims of envenomation may present the following clinical manifestations a few minutes after being stung: localized pain, hyperesthesia, hypersalivation, profuse sweating, vomiting, hyperexcitability, acute pancreatitis, gastric mucosa injury, hypertension followed by hypotension, respiratory and cardiovascular arrhythmias, cardiogenic shock, pulmonary edema, respiratory depression, acid-base disorders, and serious coagulation disturbance (Freire-Maia and Campos, 1989; Andrade et al., 2004).

The influence of *T. serrulatus* venom on the blood coagulation system was first described in 1938 (Magalhães and Tupinambá, 1938). Ensuing studies demonstrated that toxins from other scorpion species may cause diffuse intravascular coagulation, fibrinolysis syndrome, or platelet aggregation (Devy et al., 1970; Reddy et al., 1972; Hamilton et al., 1974; Longenecker and Longenecker, 1981). The epinephrine released during a scorpion accident was suggested to play an important role in stimulating blood coagulation (Longenecker and Longenecker, 1981; Beker et al., 1983), but further studies are necessary to assess all factors associated with the toxin-induced coagulopathy.

Neurotoxic peptides present in the *T. serrulatus* venom interact with voltage-gated $Na+$ and K^+ channels of autonomic system nerve endings, stimulating the neuroendocrine and immunological axis, with consequent massive release of catecholamines, acetylcholine,

* Corresponding author. Tel.: +55 3132499931; fax: +55 3132489760.
E-mail address: jrcmelo@medicina.ufmg.br (J.R. Cunha-Melo).

http://dx.doi.org/10.1016/j.toxicon.2014.10.010
0041-0101/

glucagon, cortisol, angiotensin-II, bradykinin, and prostaglandins (Vasconcelos et al., 2005; Cologna et al., 2009). These agents seem to determine tissue injury and multiple organ failure via their relation to the release of pro- and anti-inflammatory cytokines such as IL-1β, IL-1α, IL-6, IL-8, IL-10, NO, GM-CSF, TNF-α, and INF-γ (Andrade et al., 2007; Petricevich, 2010; Magalhães et al., 1999).

Excessive activation of the systemic host inflammatory response by the increase in proinflammatory cytokine production after scorpion envenomation may lead to immunological imbalance, multiple organ dysfunction, and death (Petricevich, 2010). The pathophysiology of these events is complex and not yet fully understood. It might well be possible that the strong connection between inflammation and blood coagulation play a role in this mechanism (Esmon, 1999).

Thromboelastography was developed by Hartert in Heidelberg, Germany during World War II and proved to be a reliable tool to detect changes in all phases of coagulation and fibrinolysis (Hartert, 1948; Donahue and Otto, 2005). At the turn of the century, thromboelastography underwent significant advances and is now known as rotational thromboelastometry (ROTEM) (Franz, 2009; Luddington, 2005; Wohlauer et al., 2005). Currently, ROTEM is used as a guide for hemoderivative transfusion and protamine sulfate and antifibrinolytic administrations, as well as to perform animal research on coagulation profiles, giving support to solid organ transplantation and cardiothoracic, trauma, and veterinary surgery.

1.1. Objective

The aim of the present study was to investigate the effect of a pure fraction of *T. serrulatus* scorpion venom (gamma toxin) on blood coagulation in rats via ROTEM.

2. Materials and methods

2.1. Scorpion toxin

We used gamma toxin fraction (Tx) obtained from *T. serrulatus* scorpion crude venom using a combination of water extraction and column chromatography on Sephadex G-25 (Gomez and Diniz, 1966).

2.2. Animals

Sixty-eight male Wistar rats (*Rattus norvegicus*) weighing 200–300 g were fed normal laboratory chow, given water to drink *ad libitum*, and kept in our institution's animal housing facilities. All animals were carefully monitored and maintained in accordance with ethical recommendations as approved by the Comitê de Ética para Experimentação Animal (Ethics Committee for Animal Experimentation) of the Universidade Federal de Minas Gerais – MG, Brazil.

2.3. Surgical procedures

The rats were anesthetized with intraperitoneal injections of ketamine (1.8 ml/kg) and xylasine (0.75 ml/kg) and allowed to breathe spontaneously through a tracheal plastic cannula. The left jugular veins and right carotid arteries were cannulated with polyethylene (PE) tubing PE50 heat-fused to PE10 (0.28 mm i.d.). Tx (75 μg/kg) or Ringer's lactate solution (0.15 ml/kg) was injected into the left jugular vein, which was also used for anesthesia maintenance. Blood samples (1 ml) were taken from the right carotid artery catheter using insulin syringes and collected into tubes containing sodium citrate 3.2% (Minicollect® Capillary Blood Collection Systems, Greiner Bio-One, Kremsmünster, Upper Austria, Austria) for posterior assessment of ROTEM profiles. The first sample was obtained at the beginning of hemodynamic recording (baseline) and the second sample after two, five, 15, 30, and 60 min after Tx injection. Immediately after collection of each blood sample, the carotid artery catheter was coupled to a pressure transducer (Transducer Amplifier, Model PM 1000, DATAQ Instruments, Inc. Software, Akron, OH, USA), allowing continuous recordings of mean arterial pressure (MAP).

2.4. Thromboelastometry (ROTEM)

ROTEM (ROTEM® Coagulation analyzer, Pentapharm, Munich, Germany) was performed using the homogenized, citrated blood samples pipetted gently into a disposable plastic ROTEM cup containing a recalcification reagent solution (star-tem® 20, ROTEM® System Reagent, Sunmedcare LTDA, Rio de Janeiro, RJ, Brazil).

The followings parameters were analyzed after ROTEM curve tracings using the NATEM test (Fig. 1): 1) CT (Clotting time): time in minutes from sample placement until the tracing amplitude reached 2 mm, which represents the initiation phase of enzymatic clotting factors and is thus influenced by factors VIII, IX, XI, and XII. CT is shortened in hypercoagulable conditions and prolonged in hypocoagulable states; 2) CFT (clot formation time): time elapsed between CT time and the point at which the tracing amplitude reaches 20 mm. CFT is a measure of clot kinetics and is influenced by factors II and VIII, platelet count/function, thrombin formation, fibrin precipitation, fibrinogen concentration, and hematocrit. It is shortened by increased fibrinogen level and, to a lesser extent, by platelet function, and is prolonged by anticoagulants that affect both; 3) α (alpha angle): angle between the middle line of the tracing and a tangential line to the tracing from CT to CFT. It is measured in degrees and represents the acceleration and the kinetics of fibrin formation and cross-linking. It increases with increased fibrinogen levels and, to a lesser extent, by platelet function, and decreases by anticoagulants that affect both; 4) MCF (maximum clot formation): the maximal amplitude reached after clot initiation. MCF depends on platelet concentration, platelet function, and platelet-fibrin interaction, including factor XIII activity, and reflects clot tensile strength or stiffness; 5) TPI (thrombodynamic potential index) describes the patient's global coagulation. It was proposed by Raby (Raby, 1975; Cohen et al., 1977) and may be calculated using the following equation: TPI = (100*MCF)/(100−MCF)/CFT.

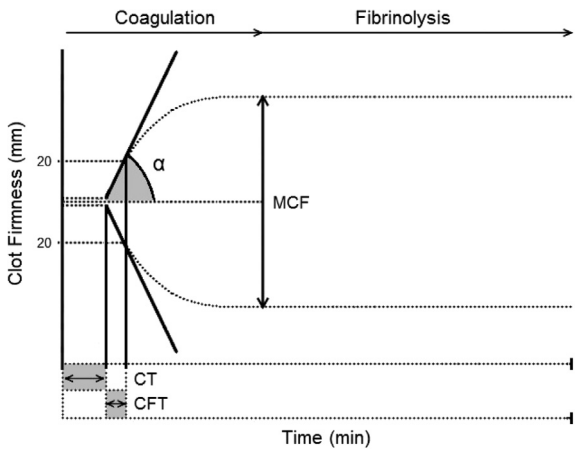

Fig. 1. Thromboelastometry tracing. The diagram indicates the main parameters analyzed that yield indices of coagulability. CT (clotting time), CFT (clot formation time), α (alpha angle), MCF (maximum clot formation).