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Toxicon 51 (2008) 1017–1028

TOXICON

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Immunochemical and proteomic technologies as tools for unravelling toxins involved in envenoming by accidental contact with *Lonomia obliqua* caterpillars ☆

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Received 15 December 2006; received in revised form 22 January 2008; accepted 28 January 2008
Available online 3 February 2008

Abstract

The accidental contact with *Lonomia obliqua* caterpillar causes local and systemic symptoms (such as fibrinogen depletion), leading, in some cases, to serious clinical complications (acute renal failure and intracranial haemorrhage). Fortunately, a successful therapeutical approach using anti-Lonomic serum, produced in horses against *L. obliqua*'s bristle extract, has already been put in place. However, a global view of immunogenic toxins involved in the coagulation disorders could help to elucidate the envenoming process. In the present study, our aim was to identify bristle extract's immunogenic components, especially those related to the haemostasis, coupling proteomics and immunochemical approaches (bidimensional electrophoresis, mass spectrometry and immunoblotting). The bidimensional map of bristle extract showed a broad profile of 157 silver-stained spots, where at least 153 spots were immunochemically revealed. Twenty-four of these spots were submitted to sequencing by mass spectrometry and three different categories of proteins were identified: lipocalins, cuticle proteins and serpins. From these protein families, it was observed that the most abundant was the lipocalin family, specifically represented by different isoforms of Lopap (a prothrombin activator protein), reinforcing its relevance during envenoming. Peptide sequences of several other immunochemically revealed spots showed no correspondence to any known sequence and were classified as unknown proteins. These proteins could represent new immunogenic molecules and/or toxins. The sequences presented in this article can be used for oligonucleotide design aiming the amplification of cDNAs coding for new molecules using *L. obliqua* bristles' cDNA libraries or isolated RNAs as template.

Keywords: Caterpillar; *Lonomia obliqua*; Bristle extract; Bidimensional electrophoresis; Immunoblotting; Mass spectrometry

☆ *Ethical statement:* All experiments were done according to good laboratory practices and to current animal manipulation techniques. All authors agree with the content and publication of the present manuscript.

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doi:10.1016/j.toxicon.2008.01.013

1. Introduction

Coagulation disorders have been reported after contact with the Saturniidae caterpillars from the *Lonomia* genus. Specifically, accidents caused by *Lonomia achelous* have been described in Mexico, Venezuela, Guiana and north Brazil (Amazonian region) (Arocha-Pinango, 1967; Arocha-Pinango et al., 2000). Since 1989, accidents involving *Lonomia obliqua* species were reported in Argentina, Paraguay, Uruguay and in the south of Brazil (Kelen et al., 1995; Zannin et al., 2003). In the last years, accidents have also been reported at other geographical areas of the Brazilian territory, such as the southeast region (Garcia and Danni-Oliveira, 2007). This organism's biological cycle is composed of 4 phases with distinct durations: egg (17 days); caterpillar (6 instars in 90 days); pupa (1–3 months) and moth (8 days) (Lorini and Corseuil, 2001). The physical contact with the Lepidoptera larvae in its 5th instar induces a toxic secretion from bristle spicules, which promotes local and systemic symptoms in the victim between 6 and 72 h after contact, such as a burning sensation, intense haematuria, disseminated intravascular coagulation-like reactions (severe depletion of coagulation factors) and secondary fibrinolysis (Zannin et al., 2003). Serious clinical complications, such as acute renal failure (Duarte et al., 1990) and intracranial haemorrhage (Kelen et al., 1995), may also occur. The envenomation process is influenced by the amount of venom injected, the instar stage, the number of smashed larvae, the extension of the skin area affected and the deepness of the injury.

The *L. obliqua* bristle extract has a complex toxic composition, from which two procoagulant proteins were already described: prothrombin activator (Lopap, ***L****onomia* ***o****bliqua* **p**rothrombin **a**ctivator **p**rotease) (Reis et al., 2001a, b, 1999) and FX activator (Losac, ***L****onomia* ***o****bliqua* **S**tuart factor **ac**tivator) (Alvarez Flores et al., 2006). Native Lopap was characterized as a 69 kDa lipocalin (isoelectric point (p*I*) around 6.0) harbouring serine protease-like activity (Reis et al., 2001b). It converts prothrombin into thrombin, by a prothrombinase complex-independent pathway, activating the coagulation system and leading to fibrinogen depletion (Chudzinski-Tavassi and Alvarez Flores, 2005; Reis et al., 2001a, b). In human umbilical vein endothelial cells (HUVECs), Lopap induced a higher expression of ICAM-1 and E-selectin, but not of VCAM-1 (Chudzinski-Tavassi et al., 2001), and stimulated the release of nitric oxide (Fritzen et al., 2005). In contrast to native Lopap, its recombinant active form was expressed in *Escherichia coli* as a monomer of about 20 kDa (Reis et al., 2006).

Another procoagulant toxin, Losac, an FX activator (~43 kDa) induces a similar cleavage pattern when compared with RVV-X, a P-IV class metalloproteinase from the venomous snake *Vipera russelli* (Chudzinski-Tavassi and Alvarez Flores, 2005). Losac is a growth stimulation agent with anti-apoptotic activity on HUVECs (Alvarez Flores et al., 2006).

Some other haemostasis-acting proteins were also identified in a cDNA library constructed upon bristle extract, including hyaluronidases, bradykinin agonist, cathepsin and phospholipase A_2-like molecules (Gouveia et al., 2005; Seibert et al., 2006; Veiga et al., 2005).

From a therapeutic standpoint, the anti-Lonomic serum, produced against the crude bristle extract from *L. obliqua* (5th instar) in horses by Instituto Butantan (Rocha-Campos et al., 2001), has been successfully used to re-establish the physiological coagulation parameters in poisoned patients. No more deaths were reported since serum therapy has been applied, according to clinical data from the Toxicological Center at ''Universidade Federal de Santa Catarina'' (CIT-SC). However, a global view of immunogenic toxins involved in the coagulation disorders could help to elucidate their role in the envenoming process.

The purpose of the present study was to highlight the main immunogenic proteic components present in *L. obliqua* venom by the use of proteomics methodologies based on their separation (bidimensional electrophoresis) and identification (mass spectrometry (MS)) coupled with immunochemical characterization (immunoblotting).

2. Materials and methods

2.1. Sample preparation

L. obliqua caterpillars from Santa Catarina (south of Brazil) in the 5th instar were anaesthetized by freezing. Their bristles were cut and ground with a mortar and pestle, in the presence of liquid nitrogen, and stored at −80 °C until use.

2.2. Two-dimensional electrophoresis

L. obliqua's bristles were extracted in the rehydration solution, which was composed of 8 M urea,