Toxicon 84 (2014) 7–18

Contents lists available at ScienceDirect

Toxicon

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

Apoptosis, mast cell degranulation and collagen breakdown in the pathogenesis of loxoscelism in subcutaneously implanted sponges

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ARTICLE INFO

Article history:
Received 6 February 2014
Accepted 5 March 2014
Available online 21 March 2014

Keywords:
Loxosceles
Loxoscelism
Sponge implants
Apoptosis

ABSTRACT

Envenomation by the *Loxosceles* spider causes loxoscelism, a pattern of signs and symptoms that primarily manifests in the dermonecrotic form. Our studies have shown that a mouse subcutaneous sponge implantation model may be useful in evaluating the effects of *Loxosceles similis* venom. This model provides an ideal microenvironment in which to study loxoscelism; however, it is still important to evaluate its pathogenesis and to observe the effects of *L. similis* venom for longer time periods than those in previous studies of this model. The aims of this study are: (1) to histologically characterize the effects of *L. similis* crude venom in a subcutaneous sponge implant; (2) to quantify the mast cells present in the implant and to measure their degranulation activity; (3) to quantify collagen subtypes I and III; and (4) to verify, quantify, and evaluate the effects of apoptosis in the implant on the pathogenesis of loxoscelism at 1 h, 4 h, and 24 h after injecting the venom. Thirty *Swiss* mice (6–8 weeks old, male) were subcutaneously implanted with polyester-polyurethane sponge discs. Fourteen days post-implantation, the animals were divided into six groups (5 animals per group): three control groups (C1h, C4h, and C24h), in which the mice received 30 μl injections of intra-implant saline, and three treated groups (T1h, T4h, and T24h), in which the mice received 30 μl (0.5 μg) injections of *L. similis* crude venom at 1 h, 4 h, and 24 h intervals. After each time interval, the animals were euthanized, and the implants were harvested and processed for light and electron microscopic analyses. The following results were observed in the implants harvested from the treated groups: acute inflammation with marked edema, thrombus, and vasculitis, as well as increased levels of mast cells and mast cell degranulation, and apoptosis in giant cells. Furthermore, degradation of collagen types I and III was observed. An analysis of the ultrastructure revealed apoptosis in various cell types. The present results suggest that apoptosis in some cell types

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http://dx.doi.org/10.1016/j.toxicon.2014.03.003
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associated with an increase in mast cell degranulation and the degradation of collagen fibers are important in the pathogenesis of loxoscelism therefore may explain the difficulty in repairing the ulcer is commonly observed in severe cases of loxoscelism cutaneous in humans.

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1. Introduction

Loxoscelism describes the lesions and clinical manifestations that result from bites caused by *Loxosceles* spiders, commonly known as brown spiders. The clinical manifestations of loxoscelism are generally characterized by dermonecrosis at the bite site (67%–100% of cases) and may be followed by systemic effects that can cause acute renal failure and, occasionally, death (Hogan et al., 2004; Da Silva et al., 2004).

The bite is initially painless, but after 2–8 h, pain, moderate to severe edema, and erythema appear at the bite site. Within 12–24 h, the area becomes pallid because of ischemia and is surrounded by a white halo. This lesion, known as marble plate, may ulcerate, and in some cases, healing difficulties may necessitate surgical excision and skin grafting (Hogan et al., 2004; Da Silva et al., 2004).

The rabbit model is most frequently used to study dermonecrotic loxoscelism (Ospedal et al., 2002; Chatzaki et al., 2012; Pereira et al., 2012b). However, a recent study by our group (Pereira et al., 2012a) showed that a mouse subcutaneous sponge implantation model (Andrade et al., 1987, 1992) was useful in assessing the effects of *Loxosceles similis* (*L. similis*) venom because the resulting lesions were similar to those observed in experimental rabbits with loxoscelism. This model mimics *in vivo* cell growth; it consists of fibrovascular tissue that contains of loose connective tissue, newly formed vessels, inflammatory cells, mast cells, foreign-body giant cells, and *Langhans* giant cells.

Although the subcutaneous implantation of sponge provides an ideal setting in which to study the loxoscelism microenvironment, it is necessary to evaluate the pathogenesis of loxoscelism and to describe the effects of *L. similis* venom for longer periods than those used in the implant model. Previous studies (Pereira et al. 2012a) have shown the involvement of apoptosis in the pathogenesis of endothelial cells in skin loxoscelism in a rabbit model. We must now determine whether the same phenomenon occurs in a fibrovascular tissue implant. This study had four aims: (1) to histologically characterize the effects of *L. similis* venom on fibrovascular tissue after the subcutaneous implantation of sponges; (2) to quantify the mast cells present in the tissue implant and to measure their degranulation activities; (3) to quantify the total collagen and collagen subtypes I and III; and (4) to verify, quantify, and assess the effects of apoptosis in fibrovascular tissue implants on the pathogenesis of this model 1 h, 4 h, and 24 h after injecting *L. similis* venom.

2. Materials and methods

The present study was approved by the *Comitê de Ética em Experimentação Animal* (CETEA) of the Universidade Federal de Minas Gerais (UFMG) (process number 229/09) and by the *Comitê de Ética no Uso de Animais* (CEUA) at the *Centro de Pesquisas René Rachou* (CPqRR/Fiocruz Minas) (approval number 93/200-1).

2.1. Venom extraction

Maceration and centrifugation, according to Silvestre et al. (2005), was used to extract venom from the venom glands of adult animals.

2.2. Preparation and implantation of sponge discs to study loxoscelism

Polyether-polyurethane sponge discs (Vitafoam Ltd., Manchester, UK) measuring 6 mm thick and 11 mm in diameter (Fig. 1A) were soaked overnight in 70% v/v ethanol. Before implantation, the sponges were washed and boiled in distilled water for 20 min. The animals (thirty Swiss mice) were anesthetized with xylazine/ketamine (1 mg/kg, Syntec of Brazil). A trichotomy of the dorsal skin was performed, and skin antisepsis was performed with iodized alcohol. The sponge discs were aseptically implanted into a subcutaneous pouch through a 1 cm dorsal midline incision, which was sutured with n°5 silk thread. The animals were maintained for 14 days in the experimentation bioterium of the *Centro de pesquisas René Rachou* (CPqRR) and postoperatively monitored for signs of infection at the operative site, discomfort, and distress. The animals were given *ad libitum* access to water and food throughout the experiment.

2.3. Venom injection

Thirty Swiss mice (6–8 weeks old, male) received injections (saline or venom) fourteen days post-implantation. The animals were divided into six groups (5 animals per group): three control groups (C1h, C4h, and C24h), in which the mice received 30 μl injections of intra-implant saline, and three treated groups (T1h, T4h, and T24h), in which the mice received 30 μl (0.5 μg) injections of *L. similis* crude venom at 1 h, 4 h, and 24 h intervals.

2.4. Implant removal and macroscopical analysis

At 1 h, 4 h, and 24 h post-injection, the animals were euthanized, and the implants were removed and analyzed

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