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Effects of small-fiber neuropathy induced by resiniferatoxin on skin healing and axonal regrowth after burn

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ABSTRACT

Background: Damage to the peripheral nervous system influences wound healing and, after a deep burn, imperfect cutaneous nerve regeneration occurs. A third-degree burn model was developed in rats combined with the use of resiniferatoxin (RTX), known to promote sensory neuropathy.

Methods: Rats were injected intraperitoneally either with RTX or vehicle. A mechanical sensory assay and the hot plate thermal sensory test were performed. The structural integrity of the sciatic nerve was assessed using transmission electron microcopy. After RTX injection, third-degree thermal burns were performed. Wound closure was monitored and samples were collected for histological analysis, immunohistochemistry and immunoblotting for neuronal markers.

Results: RTX promoted both mechanical and thermal hypoalgesia. This transient RTX-mediated sensory deficit occurred without damaging the integrity of nerve fibers and induced a significant depletion of neuropeptides in both neuronal bodies and intraepidermal nerve fibers. Although wound closure rates were similar in both groups, the kinetic of granulation tissue remodeling was delayed in the RTX group compared with control group. A significant reduction of the peripherin expression in the RTX group was observed indicating impaired axonal regrowth of small fibers within the wound.

Conclusion: Our study confirms the important roles of innervation during skin healing and the defect of nerve regeneration after burn.

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Abbreviations: α-SMA, α-smooth muscle actin; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; GAP43, growth associated protein 43; PGP 9.5, protein gene product 9.5; RTX, resiniferatoxin; TRPV1, transient receptor potential vanilloid-1.

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1. Introduction

Representing a protecting interface between our body and the external environment, the skin is a highly sensitive organ innervated by the peripheral nervous system and where specific structures express sensors permitting the perception of a wide range of signals [1]. In particular, the skin is densely innervated by different sensory nerve fiber subtypes which react to temperature variation, pain and tactile stimuli. It is also known that sensory nerve fibers are tightly implicated in a variety of cutaneous physiological and pathological processes [2]. Several clinical observations indicate that damages to the peripheral nervous system influence wound healing [3].

The local destruction of the cutaneous nerve fiber network during a burn leads to an immediate neuropathy, which is obviously more serious in the context of a full-thickness burn. Although nerve fibers may regenerate after spontaneous wound healing and/or skin grafting, abnormal nerve fiber density is often observed, resulting in the alteration of skin sensation [4]. These complications which include sensibility losses, itch, paresthesia and pain are common in the first months following the burn but often gradually decrease with time. However, depending on the anatomic site of the scar or on the injury severity, they can impact the patient's quality of life and even delay their overall rehabilitation [5].

The diabetic model induced by streptozotocin treatment is a commonly used model to study peripheral neuropathy. However, in this model, diabetic neuropathy impairs sensory, autonomic and motor nerves. Small fiber neuropathy is caused by selective damages affecting C and Aδ fibers which are the main peripheral transmitters involved in nociception. In this study, we have used our model developed in mice by administration of resiniferatoxin (RTX) [6,7]. RTX is an ultra-potent capsaicin analog that acts on transient receptor potential vanilloid type 1 (TRPV1) which is a nonselective cation channel mainly expressed in sensory C and Aδ fibers. In this study, we have first completed the description of this model using rats. In addition, we have developed a model of burn in which parameters of temperature, pressure, duration and surface were strictly monitored. By combining these two models, we have analyzed the effects of a small-fiber neuropathy induced by RTX on skin healing and axonal regrowth after burn. After RTX injection, a third-degree thermal burn was performed, wound closure was monitored and samples were collected for histological analysis, immunohistochemistry and immunoblotting for neuronal markers.

2. Material and methods

2.1. *Animals and resiniferatoxin treatment*

Experiments were performed on male Sprague Dawley rats (7–8 weeks old, 320–330 g). All animal manipulations were realized according to recommendations of the European Directive of 22 September 2010 (2010/63/EU) on the protection of animals used for scientific purposes, and the protocols of ''Establishment of a model of peripheral sensitive neuropathy'' and ''Establishment of a model of thermal burns in the rodent'' were approved by the Regional Ethical Committee (CREEAL n° 1-2013-1 and n° 09-2014-09 respectively). Animals were randomized into two groups: vehicle ($n = 22$) and resiniferatoxin (RTX) (Sigma–Aldrich, Lyon, France) ($n = 21$). RTX (185 μg/kg) and vehicle (10% DMSO) were injected intraperitoneally under anesthesia with isoflurane 7 days before burn (D-7) (Fig. 1).

2.2. *Resiniferatoxin effect assessment*

2.2.1. *Evaluation of nociceptive behaviors*

To confirm the small fiber neuropathy induced by RTX and to follow it, two sensitive tests were realized at D-8 (24 h before RTX injection), D-7 (just before RTX injection), D-6, D-1, D7, D14, D21, D28, D35 and D42 after burn (Fig. 1).

2.2.1.1. Mechanical nociception. A paw pressure analgesia meter (Bioseb, Vitrolles, France) was used for performing the Randall–Selitto test in order to determine the animal threshold response to pain induced on the paw by the application of a uniformly increasing pressure. After 10 min of acclimatization, a linear increasing pressure was applied on the right hind foot with a cut off at 300 g to avoid tissue injury. The mechanical nociception threshold corresponds to the

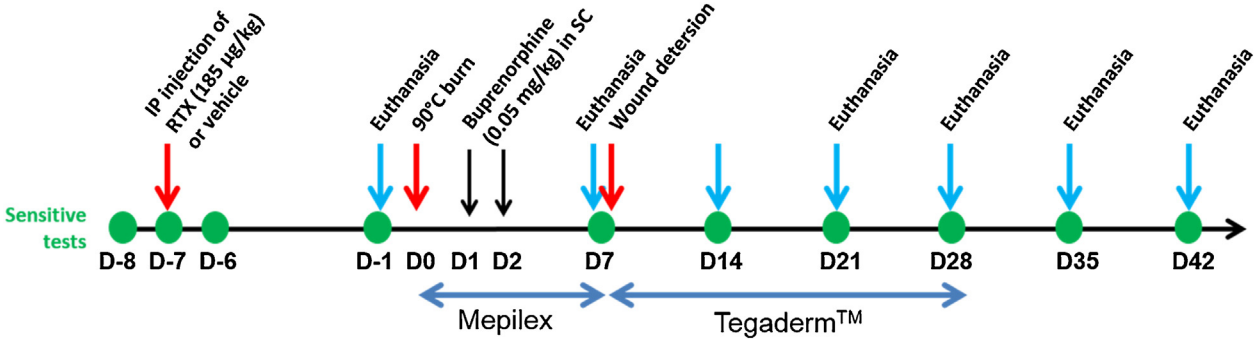

Fig. 1 – Design of the experiments. To assess neuropathy induced by resiniferatoxin (RTX), two sensitive tests were realized at D-8 (24 h before RTX injection), D-7 (just before RTX injection), D-6, D-1, D7, D14, D21, D28, D35 and D42 after burn. Euthanasia was realized at D-1 for transmission electron microscopy analysis of sciatic nerves and at D7, D14, D21, D28, D35 et D42 for dorsal root ganglia (DRGs), hind footpad, and back skin (normal or burned) sampling.

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