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Research article

Interleukin-8 levels in rat models of nerve damage and neuropathic pain

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HIGHLIGHTS

- IL-8 plays an important role in the neuropathic pain following nerve injury.
- Partial nerve injury models show elevated levels of IL-8 in the acute phase of neuropathic pain.
- Chronic constriction nerve injury shows elevation of IL-8 in the chronic phase of neuropathic pain.
- The role of IL-8 might be injury type specific.

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ABSTRACT

Interleukin-8 (IL-8) is a pro-inflammatory cytokine that has been shown to play a role in inflammatory and autoimmune disorders. The objective of the present study was to assess the levels of IL-8 in rat serum, dorsal root ganglion (DRG) and the sciatic nerve following four different forms of sciatic nerve injury. The models used to induce the injury included partial sciatic ligation (PSL), chronic constriction injury (CCI), perineural inflammation (neuritis) and complete sciatic transection (CST). Mechanical and thermal hyperalgesia were detected by measuring withdrawal responses from a mechanical stimulus and withdrawal latency from thermal stimulation. Enzyme-linked immunosorbent assays (ELISA) was used to assess the IL-8 levels in the affected and contralateral sciatic nerves. Rats exposed to PSL and neuritis developed significant nociceptive response (mechanical and thermal hyperalgesia) in the affected side at three days post-surgery whereas the CCI group at eight days post-surgery. No mechanical or thermal hyperalgesia was observed in rats exposed to CST at either three or eight days postsurgery. Additionally, IL-8 levels were significantly increased in the injured sciatic nerve at 3 and 8 days following PSL and neuritis as well as at 8 days following CCI when compared to naïve animals. A significant up regulation of IL-8 levels was observed in the ipsilateral DRG at 3 and 8 days following CST compared to naïve animals. The serum IL-8 levels remained unchanged in all models of nerve damage. The results of this study suggest that IL-8′s role in the neuropathic pain etiology may be specific to nerve injury type.

1. Introduction

Neuropathic pain is defined as “pain that arises from injury, disease or dysfunction of the peripheral or central nervous system” [1]. The mechanisms underlying the development and maintenance of neuropathic pain are not fully understood, however inflammation plays a significant role in many neuropathic pain syndromes. Following direct or indirect nerve injury, a variety of inflammatory cells accumulate at the site of injury, cascading the progression towards neuropathic pain. Neuro inflammatory mediators, along with immune responses, contribute to the initiation, development and maintenance of neuropathic pain [2]. In addition, various studies have shown that pro-inflammatory cytokines also contribute to the development and maintenance of neuropathic pain, as well as to injury induced peripheral nerve pathology [3–7]. Conversely literature has shown that anti-inflammatory cytokines have the potential to reduce neuropathic pain [8–10].

Interleukin-8 (IL-8) is a pro-inflammatory cytokine, similar to platelet factor 4, which belongs to a family of small, structurally related cytokines. It is produced by phagocytes and mesenchy-

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mal cells exposed to inflammatory stimuli (e.g., interleukin-1 or tumor necrosis factor) and activates neutrophils, inducing chemotaxis and exocytosis [11]. Recent studies show that IL-8 is involved in inflammatory diseases, persistent inflammatory pain, and persistent mechanical nociceptor hypersensitivity [12–15].

Mediators, such as prostaglandins, histamines, bradykinins and cytokines are referred to as part of the inflammatory soup and are crucial for the signaling, maintenance and initiation of inflammation and pain. Additional mediators, such as substance P and calcitonin gene-related peptide (CGRP), are usually involved in perineural inflammation. In order to understand the underlying mechanism and better target neuropathic pain, various pro and anti-inflammatory cytokines have been studied in relation to this pathological condition [3,4,10,16,17]. Interleukin-1 beta (IL-1β) and Tumor necrosis factor-alpha (TNF-α) are key to neuropathic pain, and so their relation to IL-8 in maintaining this hypersensitivity demands attention. There are limited studies that show elevation of IL-8, along with IL-1β and TNF-α, in various inflammatory conditions and nociceptor hypersensitivity [18–21]. Their importance suggests that IL-8 may share a similar function with these cytokines. The first step in understanding the role of IL-8 is to quantify these cytokines. The goal of the present study is to quantify the levels IL-8 in different types and stages of neuropathic pain. We anticipated elevated levels of Interleukin-8 in both acute and chronic stages of neuropathic pain.

2. Materials and methods

All procedures were performed according to protocol approved by Rutgers Institutional Animal Care and Use Committee, Newark, NJ and in accordance with federal law, regulations of the National Institute of Health, and guidelines of the International Association for Study of Pain (Protocol 10077E1113). Adult male Sprague- Dawley rats weighing 250300 g at the time of surgery were used in the study.

Five groups of rats were included in the study, four underwent different types of sciatic nerve injury and a group of naïve rats that were not exposed to injury served as control.

The nerve models included the Chronic Constriction Injury (CCI), Neuritis, Partial Sciatic Ligation (PSL) and Complete Sciatic Transection (CST). The rats were tested for tactile-allodynia, heat-hyperalgesia and mechanical hyperalgesia, before and up to 8 days following the procedure. Prior to Euthanasia, either on the 3rd or 8th days following the procedure, the affected and contralateral nerves, the ipsi and contralateral DRG as well as serum was collected for IL-8 levels assessment.

2.1. Behavioral assays

The rats mid plantar hind paw was tested for tactile allodynia, heat-hyperalgesia and mechanical hyperalgesia prior to surgery and on the 3rd and 8th days post-surgery. The rats were habituated pre operatively by being exposed to the sensory testing apparatus for 10–15 min for five consecutive days. During that time, the rats hind paws were tested with von Frey filaments, blunt acupuncture needle and radiant heat for heat stimuli. The examiner was unaware of the experimental groups during the testing [5].

2.1.1. Tactile allodynia

Tactile-allodynia was tested with von Frey hairs utilizing Semmes-Weinstein monofilaments (Stoelting Inc., Wood Dale, IL) [22]. This series includes monofilaments sorted by ranks expressing the log base10 of the force applied in grams to bend the filament. Three filaments numbered 4.93, 5.18 and 5.46 applying a force of 8 g, 15 g and 26 g, respectively were used. With the rat placed on the perforated floor, the monofilaments were applied 5 times at intervals of 1–4 s to slightly different loci of the mid plantar hind paw. A withdrawal response was recorded. Rats in pain elicited a higher nociceptive response rate out of the 5 stimuli.

2.1.2. Heat hyperalgesia

For heat hyperalgesia, rats were placed on an elevated glass floor platform and a high intensity moveable radiant heat (photocell) source was placed underneath the glass. Each rats plantar surface of one hind paw was exposed to the heat source. The photocell has a controlled timer which is activated when the hind paw is placed on the glass and the timer stops when the hind paw is withdrawn. The latent period of the reflex was measured from the onset of heat till the withdrawal, to the nearest 0.1 of a second. Each hind paw was tested three times with a five minute interval between each test, shorter withdrawal latency indicated more pain [23].

2.1.3. Mechanical hyperalgesia

Mechanical hyperalgesia was evaluated by measuring the duration of paw withdrawal from a blunted acupuncture needle in a pinprick test. The rat was placed on an elevated perforated floor and the tip of a 0.2 mm diameter blunted acupuncture needle was pricked against the mid plantar hind paw, until the needle slightly bent (the skin was dimpled but not penetrated). Under these conditions the bent needle exerted a mean force of 10.5 g as measured on a laboratory scale. The duration of paw nursing before it was placed back on the perforated metal floor following the pinprick evoked nociceptive withdrawal reflex was timed for up to 15 s. All the behavior testing was performed by the same investigator (HH). Normal responses of very short duration, too quick to time accurately was assigned a duration of 0.5s. An increase in the time of nursing of the paw was interpreted as an increased in the nociceptive response.

2.2. Surgical procedures

2.2.1. Anesthesia

The nerve surgery was performed on all rats using ketamine–xylazine as anesthesia (60 mg/kg and 7.5 mg/kg, intraperitoneal injection), and surgery was limited to the left sciatic nerve. After the nerve manipulation, the femoris muscle was closed with vicryl sutures, followed by clips on the outer skin to ensure proper wound closure. The rats were monitored on a daily basis.

Rats with iatrogenic damage or evidence of autotomy were excluded from the study. A total of two rats were excluded as there were signs of paralysis 24–48 h post-surgery and it may be indicative of permanent tissue damage beyond the nerve.

2.2.1.1. Chronic constriction injury (CCI). Surgery was performed according to the original developed procedure [23]. The left common sciatic nerve was exposed by a mid-thigh incision. Proximal to the sciatic trifurcation, about 7 mm of the nerve was relieved of adhering tissue, and three ligatures (4–0 Ethicon Chromic Catgut) were tied loosely around it with an interval of 1.0–1.5-mm between each suture. The sutures were tied in a manner that it did not constrict the nerve and did not arrest the circulation through the superficial epineural vasculature. The same investigator (JK) performed all the surgeries.

2.2.1.2. Partial sciatic ligation (PSL). Surgery was performed according to the original report [24]. The dorsum of the nerve was carefully relieved from surrounding connective tissues at a site close to the trochanter, just distal to the area where the posterior biceps semitendinosus nerve branches off the common