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Consomic rats parental strains differ in sensory perception, pain developed following nerve injury and in IL-1 beta and IL-6 levels

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1. Introduction

Individual variations have been noted in baseline pain sensitivity and in the propensity to develop neuropathic pain following nerve injury [1]. Genetic factors play a key role in establishing the predisposition to neuropathic pain and in determining the magnitude of the immune and inflammatory response subsequent to nerve injury [2], which in turn may dictate the evolution of mirror image neuropathic pain [3]. Consomic strains can be used to identify genetic variations. They are inbred strains containing in their genome a whole chromosome from another strain and serve as invaluable tools for investigating the genetic determinants, pathways and inflammatory contribution to chronic pain.

Previously it has been shown that in the early stages of inflammation following nerve injury, endogenous hyperalgesic mediators, including pro inflammatory cytokines, such as interleukin- 6 (IL-6), predominate while, in the later stages, interleukin-1 beta (IL-1β) may play a more significant role [4–8]. IL-1β was one of the first cytokines reported to mediate inflammatory responses involved in the generation of neuropathic pain in various animal models. Its role as a pain mediator has been firmly established in the peripheral and central nervous system (CNS) [9,10]. IL-1β levels are markedly increased in certain regions of the brain, dorsal root ganglion (DRG), peripheral nerve, and spinal cord after instances of nerve injury in the periphery [4,9,11]. Intra-plantar, intra-peritoneal, intra-neural and intra-cerebroventricular administration of IL-1β results in thermal and mechanical hyperalgesia and allodynia [12]. Exposure of neutralizing antibodies to IL-1β receptors are successful in reducing and preventing neuropathic pain behavior [12].

Similarly, IL-6 is an important pleiotropic cytokine that produces time and dose-dependent nociception [4]. IL-6 levels are increased in the sciatic nerve, spinal cord, and DRG after peripheral nerve injury [4,11–13]. Administration of IL-6 causes hypernociception, while anti-IL-6 antibodies and antibodies targeting the IL-6 receptor decrease pain related behavior [14]. In addition, IL-6-deficient mice exhibit decreased thermal hyperalgesia after carrageenan injection and after nerve injury [15]. The goal of the present study was to assess consomic rat strains' variations in response to chronic constriction injury (CCI). We evaluated the baseline sensory perception among the different consomic lines and assessed the susceptibility to and development of neuropathic pain in the CCI model, as well as IL-6 and IL-1β secretion levels from the sciatic nerve following CCI.

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2. Methods

The experiments performed were in compliance with the protocol approved by Institutional Animal Care and Use Committee of Rutgers University, NJ, USA and recommendations of the International Association for the Study of Pain (Zimmerman 1983) and National Institutes of Health guidelines for the care and use of laboratory animals as revised in 1978.

2.1. Experiment 1: sensory perception and susceptibility to neuropathic pain

2.1.1. Animals and surgeries

In all experiments, male Fawn Hooded, Dahl Salt Sensitive, Brown Norway and Sprague Dawley rat strains were used, which were obtained at 4–7 weeks of age from the vendors and housed in the animal facility for approximately two weeks. The rats were group-housed within cages in the animal facility under veterinary supervision and fed with rodent chow, reverse osmosis-treated water with a twelve-hour day night cycle. They were acclimatized to the lab conditions for one week before baseline measurements were obtained for the behavioral study. The behavioral testing was conducted near mid-photophase to reduce the effects of circadian variability [16]. Surgeries were performed by a single calibrated investigator (JK) when the rats were between 8 and 12 weeks of age, weighing 250–300 g. The investigator performing the behavioral testing was blinded to the surgical procedure performed. A total of six rats per group were included in the study. Anesthesia using intraperitoneal ketamine (50 mg/kg) and xylazine (7.5 mg/kg) solution was administered prior to the surgical procedures.

2.1.2. Chronic constriction injury (CCI)

The surgical procedures were performed in accordance with the original description [17]. In brief, the left common sciatic nerve was exposed through a left mid-thigh incision. The adhering tissue was gently freed to expose the nerve proximal to the region of the sciatic trifurcation area for about 7 mm. This was followed by the placement of three loose ligatures around the nerve approximately 1.0–1.5 mm apart using 4/0 chromic gut. Care was taken to ensure that there was a bare minimum of constriction of the nerve and that slight movement of the ligatures was feasible. The incision was closed by suturing the muscle with stainless steel clips.

2.1.3. Behavioral assays (Pain assessment)

The mid-plantar region of the rat's hind paw was tested for mechano-allodynia prior to surgery and days 1, 3, and 8 thereafter. This area corresponded to the sciatic nerve territory. Rats were habituated to the sensory testing apparatus preoperatively by placing them in the sensory testing apparatus for 10–15 min followed by behavioral testing using von Frey filaments in the hind paw area.

The technique used to conduct mechano-allodynia tests using von Frey filaments was in accordance with the methods described previously [18]. We used a series of Semmes–Weinstein monofilaments (Stoelting, Inc., Wood Dale, IL, USA) sorted by ranks expressing the log10 of the force applied in milligrams from 2.36 to 5.88 log10 g, that apply a force of 0.02–60 g, respectively. The rats were placed on a perforated floor. The mid-plantar hind paw region was tested in slightly different loci five times with intervals of 1–4 s by applying monofilaments in ascending order of their stiffness. The first monofilament that evoked, at a minimum, one withdrawal response was taken to be the threshold.

2.2. Cytokine levels in CCI

2.2.1. ELISA

On the 3rd and 8th days post-operative (DPO), rats were randomly chosen and euthanized. Ten mm of the sciatic nerve adjacent to the CCI site and a comparable ten mm of sciatic nerve on the contralateral side were harvested. Each section of sciatic nerve exposed to CCI and its comparable piece from the normal nerve, was weighed and placed separately in 2 ml of the medium for 24 h. The medium was comprised of 10% Fetal Calf Serum, 89% Dulbecco modified Eagle Medium with D-Glucose 4500 mg/l, and 1% Penicillin–Streptomycin Amphotericin B Solution. The collected medium was assayed for cytokine levels using a two-site enzyme-linked immunoassay (ELISA, R&D systems, Inc., MN, USA). The assay was conducted per manufacturer instructions and as previously reported [4]. With the help of a standard solutions curve; IL-1β, IL-6 levels were calculated in pg/mL (medium)/mg (tissue)/24 h.

2.2.2. Statistical analysis

Data was entered and analyzed using Stat View5 software (SAS Institute Inc. NC, USA). Only the rats that had data at all time points were included for analysis. Alpha (two-tailed) for significance was set at 0.05 for all of the analyses. Factorial analysis of variance (ANOVA) was used to analyze the different time points and the difference between groups was analyzed with Fishers PSLD.

3. Results

3.1. Experiment 1: sensory perception and susceptibility to neuropathic pain

At baseline, the Dahl Salt Sensitive rats, when compared to other strains, demonstrated significant sensitivity. In the tactile allodynia test for rats that underwent CCI, the threshold applied force was (4.358 ± 0.086 log10 mg) for the right hind paw, which was (1.297 ± 0.1899 log10 mg, $p < 0.0001$), (0.9953 ± 0.1612 log10 mg, $p = .0002$), and (0.9860 ± 0.2506, $p = .0045$) lower than that of the Brown-Norway, Fawn-Hooded, and Sprague Dawley lines, respectively. The left hind paw had similar results.

Following CCI, the Dahl Salt Sensitive rats developed the greatest increase in hypersensitivity, as depicted in Fig. 1. On 3 and 8 DPO, Dahl Salt Sensitive rats developed a (0.5460 ± 0.1793 log 10 mg, $p = .0159$) and (0.8900 ± 0.1890 log 10 mg, $p = .0015$) decrease in tactile allodynia threshold. By comparison, Fawn Hooded rats displayed an insignificant change on both 3 DPO (0.1533 ± 0.1672 log10 mg, $p = .3807$) and 8 DPO (0.1233 ± 0.1926 log10 mg, $p = .5398$), as did the Sprague Dawley 3 DPO (0.0400 ± 0.3022 log10 mg, $p = .8980$) and 8 DPO (0.3080 ± 0.2767 log10 mg, $p = .2980$) and Brown Norway rats 3 DPO (0.5200 ± 0.3626 log10 mg, $p = .1820$) and 8 DPO (0.7850 ± 0.3945 log10 mg, $p = .0818$) (Fig. 1).

3.2. Experiment 2: cytokine levels in CCI

3.2.1. IL-6 levels in the CCI model among different strains

Three days following CCI, Dahl Salt Sensitive and Fawn-Hooded exhibited significantly greater elevation of IL-6 secretion than the other strains, as depicted in Fig. 2. The affected nerves had a ($168{,}600 \pm 30{,}430$ pg/mg/mL, $p = .0005$) increase in IL-6 levels in the Dahl Salt Sensitive rats, and a ($160{,}100 \pm 41{,}590$ pg/mg/mL, $p = .0039$) difference in the Fawn Hooded strain. This is compared to the smaller increase of ($12{,}440 \pm 2962$ pg/mg/mL, $p = .0057$) for the Brown-Norway strains, and a (7008 ± 2699 pg/mg/mL, $p = .063$) increase among the Sprague Dawley strains. The contralateral

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