Neuroscience Letters 607 (2015) 108–113

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Tetrodotoxin suppresses thermal hyperalgesia and mechanical allodynia in a rat full thickness thermal injury pain model

Margaux M. Salas[a], Matthew K. McIntyre[a], Lawrence N. Petz[a], Walter Korz[b], Donald Wong[b], John L. Clifford[a,*]

[a] US Army Institute of Surgical Research, 3698 Chambers Pass, Bldg 3611, JBSA Fort Sam Houston, TX 78234-6315, USA
[b] WEX Pharmaceuticals Inc., Suite 420, 1090 West Pender Street, Vancouver, BC V6E 2N7, Canada

HIGHLIGHTS

- Tetrodotoxin produced analgesia for full thickness burn wound pain.
- Systemic tetrodotoxin (8 μg/kg SC) reduced thermal hyperalgesia.
- Systemic tetrodotoxin reduced mechanical allodynia.

ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form
23 September 2015
Accepted 24 September 2015
Available online 28 September 2015

Keywords:
Tetrodotoxin
Thermal injury
Thermal hyperalgesia
Mechanical allodynia

ABSTRACT

Burn injuries have been identified as the primary cause of injury in 5% of U.S. military personnel evacuated from Operations Iraqi Freedom and Enduring Freedom. Severe burn-associated pain is typically treated with opioids such as fentanyl, morphine, and methadone. Side effects of opioids include respiratory depression, cardiac depression, decrease in motor and cognitive function, as well as the development of hyperalgesia, tolerance and dependence. These effects have led us to search for novel analgesics for the treatment of burn-associated pain in wounded combat service members. Tetrodotoxin (TTX) is a selective voltage-gated sodium channel blocker currently in clinical trials as an analgesic. A phase 3 clinical trial for cancer-related pain has been completed and phase 3 clinical trials on chemotherapy-induced neuropathic pain are planned. It has also been shown in mice to inhibit the development of chemotherapy-induced neuropathic pain. TTX was originally identified as a neurotoxin in marine animals but has now been shown to be safe in humans at therapeutic doses. The antinociceptive effects of TTX are thought to be due to inhibition of Na^+ ion influx required for initiation and conduction of nociceptive impulses. One TTX sensitive sodium channel, $Na_v1.7$, has been shown to be essential in lowering the heat pain threshold after burn injuries. To date, the analgesic effect of TTX has not been tested in burn-associated pain. Male Sprague-Dawley rats were subjected to a full thickness thermal injury on the right hind paw. TTX (8 μg/kg) was administered once a day systemically by subcutaneous injection beginning 3 days post thermal injury and continued through 7 days post thermal injury. Thermal hyperalgesia and mechanical allodynia were assessed 60 and 120 min post injection on each day of TTX treatment. TTX significantly reduced thermal hyperalgesia at all days tested and had a less robust, but statistically significant suppressive effect on mechanical allodynia. These results suggest that systemic TTX may be an effective, rapidly acting analgesic for battlefield burn injuries and has the potential for replacing or reducing the need for opioid analgesics.

1. Introduction

In 2013 alone, an estimated 450,000 burn incidents were reported according to the American Burn Association (http://burninjuryguide.com/burn-statistics/). The severity of burn wounds varies, but pain is universal and is especially an issue in 2nd and 3rd degree burns. Burn wounds produce excessive pain during treatment, which can have a profound impact on the quality of life and rehabilitation. Our research is focused on improving pain care for severely burned military service members. Current management of severe burn pain, both in military and civilian populations, is primarily through the systemic delivery of opioids, causing unintended and adverse side effects [2,5]. These include cardiac and respiratory depression, and decrease in motor and

* Corresponding author. Fax: +1 21 539 1460
E-mail addresses: john.l.clifford11.civ@mail.mil, jjgdcliff@att.net (J.L. Clifford).

http://dx.doi.org/10.1016/j.neulet.2015.09.031

cognition function amongst others. In addition, extended opioid use is often necessary for the most severely burned patients, and can lead to tolerance, opioid-induced hyperalgesia, and dependence [1,17].

Tetrodotoxin (TTX), a selective voltage-gated sodium (Na^+) channel blocker, was originally identified as a neurotoxin in marine animals, where it is utilized as a defense against predators [14]. TTX has been shown to have analgesic effects in a multitude of pain models including, the hot-plate test (acute pain model), formalin test (persistent pain model), and spinal nerve ligation and sciatic nerve chronic constriction tests (neuropathic pain models) [16,18,21]. Also, TTX is currently in clinical trials as an analgesic. A recently completed phase 3 clinical trial for cancer-related pain, phase 2 clinical trial for chemotherapy-induced neuropathic pain and a number of other trials have established overall safety for humans [10,11]. The antinociceptive effects of TTX are thought to be due to stabilization of neuronal membranes via inhibition of Na^+ ion flux required for initiation and conduction of nociceptive impulses. To date, the analgesic effect of TTX has not been tested for burn pain, although TTX is known to block Na^+ channels expressed on nociceptive fibers [23], including sodium channel Na_v 1.7, which has been shown to be essential in lowering the heat pain threshold after burn injury [24].

In this study we have tested the antinociceptive effects of TTX in a rat hindpaw full thickness thermal injury pain model, which recapitulates many aspects of human burn pain [9]. We show that TTX significantly reduces thermal hyperalgesia within 2 h following administration and that this effect does not produce hyperalgesia when administered daily over several days, as is the case for morphine [15]. Also, TTX did not significantly affect motor coordination. A burn pain indication for TTX would fulfill an unmet need for an analgesic that can either replace, or reduce quantities of administered opioid analgesics. These results suggest that TTX could be developed as an effective, rapidly acting analgesic for battlefield burn injuries.

2. Material and methods

2.1. Rat full thickness thermal injury (FTTI) model

A total of 27 adult (250–400 g) intact male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were used in these experiments. Rats were pair housed in a 12:12 h light:dark cycle with *ad libitum* access to food and water. All studies were approved by the U.S. Army Institute of Surgical Research Institutional Animal Care and Use Committee and conformed to the ILAR *Guide for the Care and Use of Laboratory Animals* and all applicable Federal, DoD, state, and local regulations. The animal facility is fully accredited by AAALAC, Intl.

Rats were subjected to a hindpaw full thickness thermal injury pain protocol that was developed by our research team [9]. Briefly, animals were anesthetized with isoflurane and injury was induced using a temperature-controlled super soldering station equipped with a slanted soldering tip (RX-80HRT-5.4D; Goot, Hiroshima, Japan). The soldering tip was heated to 100 °C and placed on the right hindpaw for 30 s, producing an approximately 0.25 m^2 thermal injury on the ventral surface of the hindpaw. This procedure resulted in a partial-thickness thermal injury, which progressed to full thickness over the course of 24–48 h. To prevent infection, silver sulfadiazine (1%) ointment was applied once to the injury.

2.2. Drug treatments

TTX was prepared from proprietary lyophilized product (supplied by WEX Pharmaceuticals Inc.) under aseptic conditions on the day of administration The lyophilized product was reconstituted with sterile water and further diluted with normal saline to a final concentration of 8 μg/ml. Unused portions were stored at 4–8 °C for up to 48 h. Vehicle control preparations were made using identical lyophilized carrier brought to the same final volume. Morphine (1 mg/ml) was purchased from Hospira Inc., TTX (8 μg/kg), morphine (5 mg/kg) or vehicle was administered subcutaneously (SC) in the flank region at the times indicated in Fig. 1, for systemic treatment.

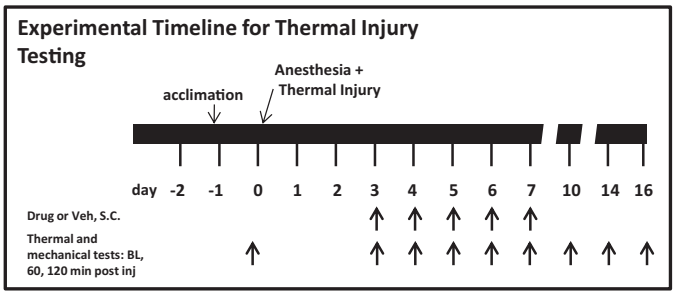

Fig. 1. Experimental timeline for thermal injury pain testing. Drug injection days (upper row of arrows) as well as thermal and mechanical testing days (lower set of arrows) are indicated. On days 0,10,14 and 16, when no drugs were administered, only baseline (BL) measurements were taken.

2.3. Behavioral testing

Nine rats were treated per group (control/morphine/TTX). All behavioral tests were performed by observers who were did not administer the treatments and who were blinded to treatment allocation. To obtain nociceptive thresholds to an acute noxious stimulus (hyperalgesia), paw withdrawal latencies (PWL) to a radiant beam of light were determined utilizing a Paw Thermal Stimulator (Univ. California, San Diego, CA, USA) as previously described [13]. Rats were acclimated to the testing apparatuses and a baseline measurement was taken prior to thermal injury. Drugs were administered daily for 5 days beginning 72 h post-thermal injury. PWLs were recorded 60 min and 120 min after each drug administration (Fig. 1). A pretreatment baseline was also taken each day immediately prior to drug administration. Nociceptive responses to a non-noxious mechanical stimulus (mechanical allodynia) were determined using a Dynamic Plantar Aesthesiometer (Ugo Basile; Collegeville, PA, USA) with automatic detection capability. For this test, the same rats used for thermal testing were placed on a mesh grid immediately following the thermal test and after a 10 min acclimation period, a blunt probe was applied to the plantar surface approximately 5 mm proximal to the thermal injury. Grams of force applied to elicit withdrawal of the hindpaw were recorded daily for 5 days beginning 72 h post-thermal injury. In addition both thermal hyperalgesia and mechanical allodynia measurements were taken on days 10, 14, and 16 post thermal injury.

2.4. Data analysis

All data were analyzed using GraphPad Prism software version 5 (GraphPad, San Diego, CA, USA). Pain behavior data are expressed as mean ± standard error of the mean (SEM) of either PWL or Force to Withdrawal. Significant outliers were identified for exclusion with the Grubbs' test (GraphPad Quick Calcs Online, the extreme studentized deviate method; [Z = (mean-value)/standard deviation], where the value is excluded if $Z > 2.21$) to detect outliers over 2 standard deviations from the mean. All data were analyzed by two-way ANOVA and Tukey's *post hoc* tests to correct for multiple comparisons. The statistical significance was tested at $p < 0.05$.