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A rat model of full thickness thermal injury characterized by thermal hyperalgesia, mechanical allodynia, pronociceptive peptide release and tramadol analgesia

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ARTICLE INFO

Article history:
Accepted 4 October 2013

Keywords:
Pain
Burn
Opioid analgesia
CGRP
Substance P

ABSTRACT

Opioid-related side effects are problematic for burn patients. Dual mechanism therapeutics targeting opioid and non-opioid mechanisms may have reduced side effects with similar analgesic efficacy. Tramadol combines mu opioid receptor agonism with norepinephrine reuptake inhibition and has been effective in treating some types of pain. The effectiveness of tramadol in treating pain associated with burns is unclear. We hypothesized that tramadol is effective in reducing thermal injury-evoked pain behaviors in a rat model. Rats were anesthetized and a 100 °C metal probe was placed on the hindpaw for 30 s to induce a full thickness thermal injury. A subset of rats was perfusion fixed and hindpaw tissue and spinal cord collected for anatomical analysis. Rats received morphine (5 mg/kg; i.p.), tramadol (10–30 mg/kg; i.p.) or vehicle and latency to paw withdrawal from a noxious thermal or non-noxious mechanical stimulus was recorded every 10 min over 70 min and again at 2 h. We report that pain behaviors developed within 48 h and peaked at 1 week; paralleled by enhanced expression of pronociceptive neuropeptides in the spinal cord. Morphine and tramadol significantly attenuated hyperalgesia and allodynia, while not significantly altering motor coordination/sedation. These data indicate dual mechanism therapeutics may be effective for treating pain associated with burns.

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1. Introduction

Blast and burn insults account for over half of modern warfare casualties [1] and advances in battlefield medical techniques, protective armor, and medical care during evacuation have led to an impressive >90% survival rate [2]. Concurrent with this improvement in battlefield survival is an increase in the number of patients needing treatment for substantive pain evoked by traumatic injuries as evidenced by a cohort of 162 soldiers receiving treatment at Walter Reed Army Medical Center who reported an average visual analogue scale (VAS) pain score of 5.9 out of 10 [3]. Burned Service Members represent one patient population in the military medical care

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0305-4179/$36.00. Published by Elsevier Ltd and ISBI
http://dx.doi.org/10.1016/j.burns.2013.10.011

system in need of optimal pain control, reduced incidence of chronic pain and reduced risk of tolerance and addiction [4].

Opioid-based narcotics are the most prevalent therapeutics for the management of severe pain in civilian and military inpatient settings [5]. Because traumatic injuries, including burns, require multiple painful treatments, including wound debridements, dressing changes and lengthy rehabilitation, tolerance to opioids resulting in dose-escalation during treatment is common [4]. This may lead to addiction, as evidenced by the near tripling of prescription drug abuse among active duty military personnel between 2005 and 2008 (Department of Defense Health Behavior Study, 2008). Reducing reliance on traditional opioid-based narcotics is one way to improve pain management and outcomes in burned Service Members and civilians.

Antidepressants that target the neurotransmitters serotonin (5HT) and norepinepherine (NE), such as amitriptyline and duloxetine, have been successful for a variety of pain conditions [6–8], with the potential added benefits of mood elevation, sleep pattern normalization and muscle relaxation. However, antidepressants have not been successful for all pain conditions and the pain-relieving properties of antidepressants alone may not be efficacious for pain experienced with severe trauma. Alternatively, pain therapeutics that target dual mechanisms simultaneously may improve pain management in this population. The dual mechanism therapeutic tramadol combines opioid receptor activation and 5HT/NE reuptake inhibition [9–11]. Both preclinical and clinical research has reported that tramadol reduces acute, postoperative, neuropathic and cancer pain [9,10,12–14] and may have a lower propensity to induce addiction [15] with little to no adverse events compared to morphine [14].

Because of the complexity and severity of pain experienced by burn patients, this population receives multiple pain therapeutics simultaneously; thus, it is difficult to determine the efficacy of a single analgesic in this population. We have developed an animal model of thermal hyperalgesia and mechanical allodynia evoked by full thickness thermal injury that shares pathological characteristics with full thickness burns in patients. We then used this model to test the hypothesis that tramadol is effective in reducing full thickness thermal injury-evoked hyperalgesia and allodynia.

2. Methods

2.1. Subjects

A total of 117 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 conform to federal guidelines and guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. This study was conducted in strict compliance with the Animal Welfare Act, implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals.

2.2. Full thickness thermal injury

A rat model of thermal injury was adapted from previous reported models [16–19] for use in these studies. Male rats were inhalation anesthetized with 4% isofluorane. Rats were laid ventrally and a 100 °C slanted soldering tip connected to a temperature-controlled super soldering station (RX-80HRT-5.4D; Goot, Hiroshima, Japan) was steadily applied to the right hindpaw for 30 s to induce a full thickness thermal injury of <1% of the total body surface area. The temperature was chosen based on previous reports [53]. Five minutes following injury and once daily for the next 4 days, 1% silver sulfadiazine cream (Watson Laboratories, Corona, CA, USA) was applied to the injured hindpaw to prevent infection. A subset of rats received silver sulfadiazine cream on the uninjured hindpaw to control for potential effects on pain behaviors.

2.3. Skin histology

Rats ($n = 4$) received thermal injury to the hindpaw and were euthanized by lethal injection of sodium pentobarbital (160 mg/kg; i.p.; Lundbeck Inc., Deerfield, IL, USA) within 5 min post-injury to examine burn depth. The collected paws were fixed in formalin, decalcified, and the plantar tissue was paraffin-embedded. The tissue was then sectioned sagittally and cross sectioned at the center of injury at 4 μM onto glass slides and stained with hematoxilin and eosin for visualization. Images were captured at 40× magnification with a Nikon Eclipse 80i microscope equipped with a DS-Fi1 camera head and depth at the focal point of injury was measured with NIS Elements Advanced Research v3.22.00 software. Three depth measurements were collected from each of 3 fields of view from the same section for a total of 9 measurements providing an average depth of the total burn area per rat. An average of three measurements taken at the deepest points of injury of each paw was also recorded. Paws were also collected at 24, 72 h, 1 week and 4 weeks ($n = 2$ per time point) post-injury to visualize burn pathology. A board certified veterinary pathologist characterized the degree of burn across the time course based on tissue morphology.

2.4. Pain behavior testing

To determine pain behaviors following thermal injury, rats were acclimated to the testing apparatuses and baseline measurements were recorded prior to thermal injury. Paw withdrawal latencies to a noxious thermal stimulus were determined using the Paw Thermal Stimulator (Univ. California, San Diego, CA, USA) as previously described [20]. For this test, rats were placed in a clear Plexiglass box resting on an elevated glass plate maintained at 30 °C. Following acclimation, a radiant beam of light was positioned under the hindpaw and the average time over three trials for the rat to remove the paw from the thermal stimulus was electronically recorded in seconds as the paw withdrawal latency (PWL). The intensity of the beam was set to produce basal PWLs of approximately 10–12 s. A maximal PWL of 20 s was used to prevent excessive tissue damage due to repeated application of the thermal stimulus.