Title
Mechanisms of pain following thermal injury

Author
Summer, Gretchen Jones

Publication Date
2005

Peer reviewed|Thesis/dissertation

MECHANISMS OF PAIN FOLLOWING THERMAL INJURY

by

Gretchen Jones Summer

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Nursing Science

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Acknowledgement

I would like to acknowledge my Dissertation Committee members. Dr. Kathleen A. Puntillo for being my Dissertation Committee Chair and my academic advisor during my doctoral program and Dr. Christine Miaskowski for being my Qualifying Committee Chair and my mentor throughout my dissertation research. I also would like to acknowledge Dr. Jon Levine, principal investigator and mentor, for his guidance, scientific input and for the many resources he put at my disposal, without which this dissertation research would not have been possible. Thanks also to Dr. Paul Green for his scientific input and to Drs. Steve Paul and Robert Gear for many helpful discussions and assistance with the statistical analyses. Finally, I would like to acknowledge the many patients with burn injuries for whom I have cared over the years. My program of research is dedicated to you in the hope that others will not suffer the way that you did.

TABLE OF CONTENTS

Introduction

Thermal injury induces a profound inflammatory response, which is manifested locally and systemically. Pain is an integral part of this response. However, little is known about the mechanisms of pain induced by a thermal burn. Clinically, pain behaviors suggest that both acute and chronic pain mechanisms are induced following thermal injury. Peripheral sensitization and activation of nociceptors by inflammatory mediators result in excruciating acute pain that is exacerbated by therapeutic procedures. Profound edema associated with inflammation distends the tissues, increasing pressure that results in compression, transection, and ischemia of peripheral nerve fibers. Neutrophils and macrophages accumulate to clean up wound debris, prevent infection and participate in wound healing. Two to three days later, Wallerian degeneration ensues as part of this multi-faceted wound healing, yet grossly inflammatory, process.

Much work has been done to define the mediators of inflammation and their origins. However, research that examines the mechanisms of pain following burn injury has not received the attention it deserves. To be sure, the models and laboratory assays necessary to tease apart the complex mechanisms that contribute to burn pain have not been well developed. However, understanding the molecular events that occur in cells subjected to noxious heat is critical to the advance the development of therapeutic targets for analgesic therapies following thermal injury. This dissertation establishes a program of research to investigate the mechanisms of pain following thermal injury. Section I provides a review of the problem of burn pain, the underlying mechanisms associated with burn injury that may contribute to burn pain, the types of pain associated with burn injury, and pain management strategies across the continuum of burn care. Section II

describes an animal model that was developed and tested to investigate the mechanisms of primary mechanical hyperalgesia induced by a thermal burn. The model demonstrates for the first time the profound intensity and long duration of thermal burn-induced hyperalgesia over the course of one week. The model is important because it allows for the continuous observation in real time of behavioral changes in nociceptive threshold that occurred before, during, and after exposure to a thermal burn. Section III describes a series of experiments that were conducted using this model to investigate the contribution of two mediators of inflammatory pain: nerve growth factor (NGF) and protein kinase C-epsilon (PKCε). Findings from these experiments suggest that NGF and PKCε each play an important role in the mechanisms by which thermal burns induce hyperalgesia. Future research will investigate these and other mediators of acute pain following burn injury as well as those that may lead to chronic pain states. The investigation of the mechanisms of pain following thermal burns is a priority program of research that has significant clinical relevance.

Section I of this dissertation is in preparation to be submitted as a manuscript for publication.

I. Burn Pain: A Severe and Difficult Pain Management Problem

Abstract

Over ten years ago, the nursing leadership of the American Burn Association ranked pain as the highest research priority because burn nurses know all too well that burn pain is difficult to manage [1]. Unfortunately, burn pain continues to be a difficult problem. Current analgesic therapies are limited in their ability to target the specific mechanisms that contribute to burn pain. While opioids are the pharmacologic agent of choice for the management of moderate to severe burn pain, they are difficult to titrate to effect in this patient population for several reasons.

Initially, most patients are opioid naïve, and, thus, may be sensitive to opioids. However, their pain may rapidly become 'resistant' to opioids. In particular, higher doses of opioids may be required to adequately manage burn procedural pain, but it is not always feasible to provide the close monitoring that these patients require. In addition, opioids may be difficult to titrate in the burn care setting where lack of skin integrity and the risk of infection may limit intravenous access. In addition to opioid analgesics, sedative/hypnotics may be indicated for severe procedural pain, but specialized personnel are needed to administer agents such as propofol or ketamine. Moreover, the adverse effects that high doses of opioids may induce can be as problematic as the pain that they are intended to manage.

Thus, the risk of opioid overmedication or undermedication in this vulnerable patient population is high. Research is needed to elucidate the mechanisms that contribute to burn pain. Only by identifying the mechanisms involved, can new therapeutic agents be developed to specifically target the mechanisms that contribute to the intensity and unpredictable variability of burn pain over the phases of burn recovery.

Introduction

Undertreatment of burn pain remains a significant clinical problem despite the wide availability of pain management information [2-11] and the promotion of guideline-based treatment approaches [12-15]. A major reason why burn pain is such a difficult problem to manage is that the intensity of burn pain is highly variable over time, both within and between individuals [16-18]. Moreover, the mechanisms that contribute to the extreme variability of burn pain are poorly understood [19]. The purpose of this paper is to provide a review of the problem of burn pain, the underlying mechanisms associated with burn injury that may contribute to burn pain, the types of pain associated with burn injury, and pain management strategies across the continuum of burn care.

Incidence, Risk Factors, and Mechanisms of Burn Injury

Incidence of Burn Injury

Aggressive prevention strategies, including legislation that required the use of smoke detectors, have significantly decreased the overall incidence of burn injuries over the past 40 years. Nonetheless, burn injury remains a significant public health problem. The magnitude of this problem is suggested by the fact that in the United States (U.S.) alone, an estimated 1.25 million individuals sustain a burn injury each year [20]. Of these, between 700,000 and 827,000 [21] seek care at an emergency department (ED), and between 51,000 and 71,000 require hospitalization. The age-adjusted death rate for burns in the U.S. has declined to approximately 4,500 to 5,500 deaths annually [22-27]. Despite these improvements, the U.S. has the highest per capita burn death rate of any

industrialized nation in the world, two to three times that of most European countries [28].

This problem is highlighted by the fact that the number of admissions to the nation's 125 specialized burn treatment centers has more than tripled (i.e., from 13% to 50% of all hospitalized burn patients) [29,30]. While this is a positive development because it demonstrates increased recognition of the importance of specialized care following a burn injury, burns are one of the most resource-intensive of all health care conditions. Therefore, there is a growing trend to treat larger and more complex burns on an outpatient basis. This approach means that outpatients experience more severe pain and have pain management needs across all phases of burn recovery.

Risk Factors for Burn Injury

Risk factors for burn injury in the U.S. include age, with children being most at risk for scald and immersion injuries, and the elderly for flame burns; gender, with males sustaining burns at least twice as frequently as females; medical conditions associated with physical or mental illness; and the use of substances that alter coordination and perception or contribute to careless smoking behaviors (e.g., drugs, alcohol, medications such as tranquilizers, and lack of or inadequate seizure medication) [28,31]. Males may sustain more burn injuries than females because males in general handle more flammable materials (e.g., gasoline and chemicals) and engage in higher risk occupations such as electrical engineering, food-service, firefighting, and military service. However, females with thermal injuries made up an estimated 43% of the nation's ED visits in 1999 [21], which suggests that the gender gap may be closing. The most common type of burn injury is a scald, which accounts for more than 100,000 ED visits per year [32].

Mechanisms of burn injury and the effects of noxious heat are described briefly in the next section.

Mechanisms of Burn Injury

Burn injury results in tissue damage caused by flame, scald, electricity, radiation, or chemical agents [19]. The two mechanisms that contribute most significantly to burn injury are temperature and duration of exposure. Temperature in the form of heat is transferred to the body through conduction, radiation, and convection (Table 1). Noxious heat is lethal to living tissues, with cell membranes being the most vulnerable [33] because they are held together by forces of hydration [34]. Temperatures of as little as 6°C above normal body temperature (i.e., 43°C) can result in the destruction of cell membranes [35]. Between 40°C and 44°C (104°F to 110°F), cellular enzymatic reactions begin to slow down, followed by failure of cellular sodium membrane exchange, which results in high levels of intracellular sodium.

Duration of exposure is a critical factor in the mechanisms of burn injury. For every degree rise in temperature between 41°C and 51°C, the time required to produce epidermal necrosis is reduced by approximately 50% [36]. Exposure of the skin to 60°C (140°F) for more than one second causes a partial-thickness burn (i.e., epidermal sloughing), and temperatures above 70°C (158°F) cause dermal protein to be denatured, producing a full-thickness burn [37]. Thus, the development of sensory sensitivity to extreme temperatures (>43°C and <15°C) have been a fundamental protective adaptation of organisms throughout evolution [38,39].

Classifications of Burn Injury

Severity of Burn Injury

The American Burn Association (ABA) has classified severity of burns into three categories: minor, moderate, and major (Table 2). The size, depth, and location of a burn injury, as well as age, pre-injury medical conditions and concomitant trauma (e.g., inhalation injury, head injury) all contribute to the severity of a burn injury. The size of a burn is usually assessed in percent (%) of total body surface area (TBSA). Smaller burns of $\leq$10% TBSA accounted for 54% of all burn center admissions in the U.S. in 2002, more than double the 26% estimated to have been admitted with a small burn prior to 1965 [40]. In contrast, the frequency of severe burns of $\geq$60% TBSA has decreased 6% since 1965, accounting for 4% of all burn center admissions in the U.S. in 2002. These figures reflect progress in burn prevention and underscore a growing awareness that burns require specialized facilities and experience regardless of size [29,30].

Depth of Burn Injury

Depth of burn is critical to a discussion of pain following burn injury because the nociceptive structures that transmit pain innervate the skin at varying depths. Nociceptors are a sub-population of primary afferent neurons that respond to noxious stimuli (i.e., tissue damage) [41]. Burns are classified by depth of injury into two broad categories: partial-thickness burns and full-thickness burns. Burn injuries are further classified within each of these categories by assessing whether the depth of injury appears to be 'superficial' or 'deep' [42-44]. Superficial partial-thickness burns may damage only the outer layer of the skin, the epidermis, but they do produce pain, particularly if touched (e.g., sunburn). [45]. These burns have a characteristic erythematous appearance and may

be swollen. If they develop blisters, they are small and develop several hours after injury [46].

Deep partial-thickness burns have a characteristic erythematous, blistered appearance. Deep partial-thickness burns develop blisters due to dilated, congested capillary loops and postcapillary venules in the most superficial part of the dermis that leak plasma [36,47]. While deep partial-thickness burns are painful, the intensity of the pain may be variable. For example, the patient may have no response to a sharp punctate stimulus such as a pinprick, but may complain of a deep achy pain [45]. In a superficial burn, tissue blanches in response to pressure, whereas in a deep partial-thickness burn, it remains erythematous.

Initially, full-thickness burns have a characteristic dry, pale, leathery appearance. Full-thickness burns, although more severe than deep partial-thickness burns, are often painless. This is due to the complete destruction of the dermis which contains nociceptive structures that transmit pain. That being said, patients often complain of pain from deep full-thickness burns, particularly as healing progresses. This clinical finding may be due to the exposure, severing, and damage of nociceptive fibers following excision of 'eschar' [19]. Eschar is devitalized tissue that initially covers the burn wound. Due to its adverse immune effects, the prompt removal of eschar, combined with timely closure of the burn wound, accounts for the significant improvement in outcomes for burn patients over the past 40 years [48-50]. Thus, early excision and grafting are the standard of care following a deep burn.

Both superficial partial-thickness and full-thickness burns are easier to identify than deep partial-thickness burns. Burns usually vary in depth considerably within the

same injury [51]. In addition, the progressive inflammatory response initiated by burn trauma triggers the release of vasoactive mediators that extend the ischemic borders and depth of a burn injury due to extravasation and hypoxia [52-55]. While the mechanisms of pain induced by thermal injury remain to be elucidated, inflammation, which is necessary to accomplish healing, appears to be the primary source of pain in burn-injured patients as described below.

Mechanisms of Burn Pain

Research suggests that the same mediators that initiate and accomplish wound healing may contribute to pain induced by burn injury. [54,56-61] (For review see [62]). Burn pain varies widely as the healing burn wound undergoes sequential phases of inflammation, repair, and remodeling. Surgically-induced trauma models have been used to describe the phases of wound healing, but burns are uniquely different in both the direction and cellular mechanisms of injury [63]. Burn wounds are characterized by heat-induced tissue coagulation [64], and unlike incision wounds, the principal direction of tissue injury following thermal burn is horizontal, not vertical [65]. These differences influence the mechanisms and the duration of pain that characterize these two distinct types of traumatic injuries.

Whereas, surgically-induced wounds heal rapidly by blood clot formation, reepithelialization, and fibroblast proliferation; cutaneous burn wounds heal more slowly, due to edema, extensive necrosis, and relative hypoxia in the burn wound [63,65]. Thus, postoperative pain gradually subsides as healing progresses, whereas the pain of burns

often worsens unpredictably over the course of healing, an event that is difficult to plan for and most distressing to patients [2,4,16,66,67].

Virtually every inflammatory mediator that has been attributed to increased vasodilation, extravascular osmolarity, and microvascular permeability following thermal burns has been implicated in pain [68-71]. However, these inflammatory mediators primarily have been studied in isolation under experimental conditions, creating an artificial environment for the inflammatory process. In reality, these mediators co-exist in a dynamic 'soup' of interactions, producing results that differ from those induced by a single mediator acting alone [72]. For example, prostaglandins are known to potentiate the effects of inflammatory mediators, such as amines [73,74], serotonin [75,76], bradykinin [77,78], and hydrogen ions [79,80] on primary afferent nociceptors.

Thus, research is needed to correlate changes in burn pain intensity with changes in the presence of inflammatory mediators in the healing burn wound over time. Studies that focus on the mechanisms of pain lag far behind studies that have focus on the mechanisms of extravasation induced by inflammation following burn injury. However, this literature offers many insights into potential mechanisms of pain following thermal injury. For example, heat-sensitive receptors (e.g., transient receptor potential vanilloid-4 [TRPV4]) were found to induce pain in response to changes in osmolarity associated with inflammation [81-84]. Yet, virtually nothing is known about the role that these channels play in generating pain following thermal injury.

While the mechanisms that contribute to burn pain remain largely unknown, some mechanisms can be inferred based on our understanding of some of the mechanisms for acute and chronic pain. For example, it is well established that inflammatory mediators

released in response to tissue injury increase the excitability of the peripheral and central (i.e., spinal cord and brain) nervous systems through mechanisms known as *peripheral* and *central sensitization.*

Peripheral Sensitization

Peripheral sensitization results in the clinical manifestation of *hyperalgesia*, a lowering of the pain threshold and an enhanced response to suprathreshold stimuli. Lewis (1935, 1942) defined two types of hyperalgesia: *primary hyperalgesia* and *secondary hyperalgesia* [85,86]. Primary hyperalgesia refers to a sensitization process that enhances "pain" transmission via a peripheral mechanism at the site of injury [87]. For example, the inflammatory mediator, prostaglandin, which is synthesized in the setting of inflammation, acts on the terminal endings of primary afferent nociceptors to make them more sensitive. Sensitization of nociceptors in this manner results from a peripheral mechanism. Secondary hyperalgesia, on the other hand, refers to a sensitization process that occurs because of changes in the spinal cord [87]. The majority of inflammatory mediators that contribute to primary hyperalgesia have been found in blister fluid or plasma following burn trauma [56,68,69,88-93]. Primary mechanical hyperalgesia is the most challenging clinical pain management problem following burn injury. Research is needed to examine how these mediators activate the pathways that contribute to burn pain and how changes in their tissue levels affect changes in the intensity of burn pain over time.

Another mechanism for burn pain that needs investigation is neurogenic inflammation. Burn injury stimulates an inflammatory neurogenic response that includes the release of Substance P and other pain producing neuropeptides from the peripheral

terminals of nociceptors [61]. Substance P is a noxious inflammatory mediator expressed by nociceptors in the periphery and in the spinal cord. During inflammation, receptors for Substance P rapidly increase their expression in the dorsal horn of the spinal cord [94,95]. These and other changes are capable of transforming not only the signals (i.e., action potentials), but the nature and function of sensory neurons. This process is called *neuroplasticity* and refers to the fact that the nervous system changes in response to nociceptive stimulation. A form of neuroplasticity that contributes to chronic pain syndromes is central sensitization, which is described in the following section.

Central Sensitization

Central sensitization results from the strengthening and reinforcing of nociceptive signals in the dorsal horn of the spinal cord [96]. That is, prolonged and substantial cutaneous receptive field changes are produced by brief noxious stimuli conducted by primary sensory neurons from the periphery to the spinal cord [97-99]. By modifying the response properties of neurons from the spinal cord, central sensitization is responsible for at least some of the changes in mechanical sensitivity that occur at the site of injury (i.e., primary hyperalgesia) and all of the changes in the area surrounding the injury (i.e., secondary hyperalgesia) [100-102]. These changes result in a notable reduction in the intensity of stimuli necessary to evoke pain so that stimuli that would not normally produce pain begin to do so. This process is referred to as *allodynia*. Central sensitization is mediated primarily by neurotransmitters such as substance P and excitatory amino acids that are released by sensory neurons in response to nociceptive stimuli. Recent studies involving ketamine and dextromethorphan, which are antagonists to the receptors for the excitatory amino acid N-methyl-D-aspartate (NMDA), have demonstrated that

NMDA receptors play a role in central sensitization following experimental burn injury [103-105].

Another form of central sensitization is called *temporal summation.* Temporal summation is evoked by prolonged repetitive stimulation of nociceptive input to the CNS [106-109]. Temporal summation of pain occurs when repeated stimuli become increasingly painful in spite of unchanged stimulus intensity. That is, temporal summation can be quantified as the difference in pain between the first and the last stimulus in a train of stimuli (i.e., action potentials). Experimental studies of volunteers undergoing burn injury indicate that temporal summation may contribute to hyperalgesia following thermal injury [110]. For example, high intensity noxious stimuli induced by frequent stimulation of the injured site during dressing changes may induce temporal summation, contributing to an increase in the intensity of burn pain. However, this has not been well investigated due to a limitation in the models available for examining the mechanisms of thermal burn-induced hyperalgesia.

Nerve damage may also contribute to an increase in the intensity of burn pain over time. Immediately following burn injury nerve endings in the skin are damaged, which evokes high intensity signaling to the CNS from the damaged skin in the periphery. In deep partial-thickness burns, thermal injury causes primary afferent axons to swell, degranulate, and demyelinate [36,111,112]. Macrophages invade the degenerating nerve stump, removing myelin and axonal debris, but leaving the basal lamina intact through a process known as *Wallerian degeneration* [113].

As damaged neurons regenerate, they may develop abnormal ectopic excitability at or near the site of nerve injury. The mechanisms responsible for these abnormalities

may include unusual distributions of sodium (Na^+) channels, as well as abnormal responses to endogenous pain producing substances and cytokines such as tumor necrosis factor-alpha (TNF-α) (For review see [114]). Persistent abnormal excitability of sensory nerve endings in a neuroma is a mechanism for stump pain induced by amputation. A *traumatic neuroma* is the term given to the mass of nonneoplastic Schwann cells and neurites that may develop at the proximal end of a severed or injured nerve [115]. Local nerve injury tends to spread to distant parts of the peripheral and central nervous systems. For example, damage to neurons in the periphery has been found to contribute to changes in the spinal cord that lead to neuropathic pain [116]. It follows that damage to sensory neurons (e.g., formation of traumatic neuromas and microneuromas) may be a mechanism for burn injury-induced neuropathic pain, but this question has not been well studied and warrants further investigation.

Excision (i.e., tangential and fascial) of eschar associated with deep burns causes intact peripheral afferent nociceptors to become injured and divided. It also seems likely that neurons regenerating from the wound margins may contribute to burn pain. Research is needed to study these potential mechanisms and their affects on the intensity of pain across the phases of burn wound healing, described further in the following section.

Pain Associated with the Phases of Burn Wound Healing

Pain is the most common symptom reported by patients following burn trauma [117,118]. The intensity of burn pain varies across the phases of burn recovery and, at its extreme, has been described as excruciating [119] and overwhelming [120]. There are three phases of burn recovery: 1) the acute (also referred to as the emergency or resuscitative phase), 2) the healing phase, and 3) the rehabilitation phase. The length of each phase varies considerably, from one day to years of hospitalization and up to months or years of rehabilitation. Factors that affect the length of each phase are patient participation in the plan of care; patient age; depth, location and size of burn as well as any comorbid or preexisting conditions (For review [121]). The pain of burns is highly variable and cannot be predicted based on factors such as patient age, sex, ethnicity, education, occupation, or socioeconomic status [16,122,123]. Moreover, burn size frequently does not correlate with burn pain intensity [123]. During each of the phases of burn recovery, pain management is directed at managing three types of burn pain: procedural pain, background pain and breakthrough pain [2,15].

Procedural Pain

Procedural pain is experienced when the wound is touched or moved and is due to primary and secondary mechanical hyperalgesia. Patients describe procedural pain as burning and stinging, with intermittent sharp pain that may continue after dressing changes and exercises have ended. Of all the types of burn pain, procedural pain is the most intense and the most likely to be undertreated [119]. Aggressive wound debridement, dressing changes, and strenuous physical and occupational therapy that require manipulation of already inflamed tissue may contribute to increased pain and

inflammation in burn wounds. However, this framework has not actually been tested; it is based on clinical experience and requires study.

Procedural burn pain often evokes anxiety and emotional distress, which may contribute to the development of long-term psychological disorders such as post traumatic stress disorder (PTSD) [124-126]. However, patients suffering from PTSD often exhibit a flattened affect, making them less likely to report their pain or to express it behaviorally [127]. Thus, clinicians may assume that the patient's pain is under control. This is of concern because PTSD is a common outcome following burn injury, occurring in approximately 30% to 50% of patients [128]. Therefore, *all* burn-injured patients must be asked to rate their pain for an accurate assessment [127,129]. It cannot be overstated that the pain of burns can only be managed with an accurate assessment, which—due to the extreme variability in intensity of burn pain—must be made frequently and well documented to insure continuity of care over the changing course of burn recovery.

Background Pain

Background pain is less intense and of longer duration than procedural pain. Wide variability has also been documented in the intensity of burn background pain [17,18]. Burn background pain is typically described as a continuous, achy type of pain that is present when the patient is relatively immobile following burn injury. Therefore, it is usually best treated with regularly scheduled analgesics that provide a continuous serum therapeutic blood level.

Breakthrough Pain

Breakthrough pain occurs when serum blood levels of analgesics drop below what is needed to control background pain. Breakthrough pain is associated with primary

mechanical hyperalgesia because it is evoked by movement, particularly in patients who have been immobile for extended periods of time.

For optimal analgesia following burn injury, a guiding principle is that each individual patient requires *separate* assessments of procedural, background, and breakthrough pain throughout the rapidly changing course of burn recovery. The next section describes the three phases of burn recovery and how these different types of pain may occur in and interfere with each phase.

The Acute Phase

Stabilization of the patient and preservation of function are the primary goals of treatment during the acute phase. Larger thermal injuries (>15% TBSA) evoke profound systemic fluid shifts and physiologic stress responses. This phase varies in length, but typically lasts no longer than 72 hours [15]. Acute intoxication with alcohol or chemical substances may initially complicate care of the burn-injured patient and can adversely interact with analgesic and anxiolytic medications [15].

Procedural Pain During the Acute Phase

Procedural pain during the acute phase may range from mild to excruciating. It is critical to aggressively manage pain and anxiety for the first dressing change. If the first dressing change evokes extreme anxiety and emotional distress, clinical experience suggests that these responses are likely to increase over time and may lead to long-term pain management problems [11]. Thus, some burn centers provide anxiolytic medication as an adjunct to opioids as a standard of care during this phase unless otherwise contraindicated [130].

Of note, patients may have progressed to the healing or rehabilitation phase and then return to the acute phase following surgery. Thus, the phases of burn recovery should not be considered mutually exclusive and do not always occur in a step-wise progression.

Background Pain During the Acute Phase

Background Pain during the acute phase may range from none at all to severe. Background pain is often markedly decreased by covering the wound securely with a moist or occlusive dressing. Because background pain is continuous, slow release opioids or analgesics with a long half-life such as methadone are particularly effective for the management of background pain [131]. Moreover, methadone is a mu-receptor agonist and NMDA-receptor antagonist. Thus, it may be of particular benefit to decrease morphine tolerance, which is often associated with hyperalgesia in the burn-injured patient [19,132]

Breakthrough Pain During the Acute Phase

During the acute phase, it is not unusual for the burn-injured patient who appears to have good pain control on a stable analgesic regimen to suddenly develop acute pain, referred to as breakthrough pain. A sub-type of breakthrough pain called end-of-dose breakthrough pain may occur toward the end of a dosing interval presumably because opioid levels have declined below an optimal level. Usually shortening the dosing interval is recommended under these circumstances [133]. However, increasing the dose without shortening the dosing interval may also be effective, but this approach may result in higher than desirable peak serum levels that can produce or increase undesirable side effects [134].

The Healing Phase

During the healing stage, the goal is to lift devitalized tissue from the injured area to promote healing. This goal is usually accomplished with repeated wound debridement or surgical excision. During the transition from the acute to the healing phase, a determination is made as to whether the patient will require surgery to accomplish wound closure or 'to let the wounds heal on their own' with daily wound care. It is necessary to determine burn depth before this decision can be made. Since it was established that early excision and grafting significantly reduce mortality and morbidity in the burn-injured patient population [135], the goal is to establish a treatment plan as soon as possible. That being said, a determination of the depth of tissue damage is difficult because inflammatory responses induce changes in the local microcirculation that contribute to further ischemia. Thus, the extent of tissue damage does not evolve completely for several days (e.g., three days or longer) following burn injury [136]. During this 'wait and watch' period of time, the burn patient often experiences severe pain and anxiety.

Procedural Pain During the Healing Phase

Unlike most acute injuries, procedural burn pain may worsen unpredictably over the course of healing, an event that adds to emotional distress in this patient population [2,4,16,66,67]. This problem is not limited to the healing phase, but is a frequent clinical observation, particularly during the healing phase. Moreover, the healing phase can last a long time—weeks, months, and sometimes even years—during which time, the patient must endure repetitive, exquisitely painful, wound care procedures.

Background Pain During the Healing Phase

Background pain during the healing phase is often associated with movement. Due to the critical need for mobilization of the burn-injured patient, effective management of background pain is critical to minimize the risk of complications (e.g., respiratory infection, deconditioning, loss of function, and contractures). As previously stated, regularly scheduled administration of medications that provide continuous therapeutic serum blood levels is indicated for the management of background pain during this phase of burn care. Several doses of systemically administered medication are required before a therapeutic serum level is achieved. Thus, a 'loading dose' may be indicated. Clinical experience suggests that background pain is less intense than either procedural pain or breakthrough pain, but research is needed to characterize this type of pain and the variability of its intensity over the phases of burn recovery.

Breakthrough Pain During the Healing Phase

A common clinical observation during the healing phase is that breakthrough pain may increase precipitously before reepithelialization is complete. Increased pain characteristically occurs with the appearance of epithelial skin 'buds', structures that herald reepithelialization and subsequent wound closure. This period of healing is exquisitely painful, but the pain ceases rapidly as epithelial cells spread out to cover the burn wound. The time course of angiogenesis, neural regeneration, and reepithelialization during the healing phase of burn recovery warrants investigation in correlation with changes in primary mechanical hyperalgesia, the major source of severe pain in burn-injured patients.

The Rehabilitation Phase

The rehabilitation phase is characterized by completion of wound closure, scar tissue maturation (i.e., remodeling), and aggressive physical and occupational therapy to prevent contractures and optimize functional outcome. During the rehabilitation phase, complaints of paresthesias, dyesthesias, cold allodynia and neuropathic pain may arise [137-139]. In a study of 236 patients who responded to a survey (67% response rate) at least one year following burn trauma, over one-third of participants complained of pain. A total of 71% complained of noxious paresthetic sensations. Only 28.4% reported no sensory problems [138].

In another study, cutaneous sensory dysfunction and subjective reports of abnormal sensations were compared between patients with healed burns and uninjured volunteers [140]. Tactile, thermal, and pain thresholds were measured in the upper extremities of 121 patients with healed burns (i.e., more than 18 months following burn injury) and pair-matched to 121 healthy controls. Deep burn sites that required skin grafting showed a significantly higher percentage of abnormal thresholds than corresponding sites in healthy, control subjects ($P<0.0001$). Moreover, the majority of the patients who had very abnormal thresholds reported painful or paresthetic sensations in the previously injured site. Depth of burn was the only factor associated with the severity of sensory deficit, explaining more than one-third of the observed variability. Patients' age, gender, and medical variables, such as size of burn or time elapsed since the injury did not explain a significant proportion of the variation in the sensibility thresholds in this study.

Few studies have examined neural regeneration in human autograft [141] and healing skin [60,142] following burn injury. Of these, only one obtained comparison samples, making it possible to determine changes over time [141]. Clearly, the study of neuropathic pain following burn injury is a high priority for future research.

Procedural Pain During the Rehabilitation Phase

Because the pain management needs of patients with burns usually decline markedly once healing has occurred, short-acting oral opioids are indicated for procedural pain during the rehabilitation phase. For severe pain, a strong opioid (e.g., fentanyl, hydromorphone, morphine) may be indicated. For mild to moderate pain, oxycodone or a similar opioid preparation mixed with acetaminophen is indicated. Opioid medications should be discontinued as indicated during this phase. Due to the frequent need for large doses of opioid analgesics up until the day that wound closure is accomplished, tapering of opioids may be indicated to avoid symptoms of withdrawal. Virtually no research has been done to study this issue, to identify at risk patients, or to make recommendations for opioid tapering schedules that are safe and manageable.

Background Pain During the Rehabilitation Phase

Background pain is usually not a problem during the rehabilitation phase of burn recovery. If it is, however, the same principles as suggested for treating background pain during the healing phase should be followed.

Breakthrough Pain During the Rehabilitation Phase

As background pain is usually not a problem, it follows that breakthrough pain is usually not a problem during the rehabilitation phase of burn recovery. There may be

pain associated with a painful incident such as movement or stretching exercises, but this type of pain can often be anticipated and planned for as a painful procedure.

Clinical Management of Burn Pain

The Acute Phase

The acute phase of burn recovery involves the admission and postoperative periods, during which the first doses of opioid analgesics are often given. The management of procedural pain and anxiety in patients with burns is a challenge that requires striking a balance between inadequate versus excessive medication. During the acute phase, variability of burn pain intensity is a particularly difficult problem. This problem is exacerbated by the fact that during this phase patients are typically opioid-naïve. Thus, they may be sensitive to more than a moderate dose even though their pain is often severe. To add to the complexity of pain management during the acute phase, clinical experience suggests that burn pain becomes rapidly 'resistant' to opioids, even at very large doses [2]. Based on the reported opioid sensitivity of chronic noncancer pain [143], Choinière suggested that opioid resistance may be a symptom of the development of neuropathic pain in burn-injured patients [2]. This theory, which has not been tested, warrants further investigation.

Moreover, the use of opioids often produces a variety of side effects that impede recovery of the burn patient, most frequently nausea, sedation, immunosuppression, hypotension, loss of appetite, and dysphoria [9,131,144,145]. The severity of these side effects often limits the use of opioid analgesics [146-148]. Thus, the risk of overmedicating or undermedicating burn-injured patients during the acute phase is high.

Clinical Management of Procedural Pain During the Acute Phase

Morphine is the most frequently administered opioid for procedural pain in burn-injured patients, but other opioids such as hydromorphone, fentanyl, and nalbuphine are also recommended [131]. Opioids should be administered intravenously or orally, not intramuscularly. Of note, intravenous (IV) access may be contraindicated in some burn-injured patients, particularly when the risk of infection (i.e., sepsis) outweighs the benefit of IV access for analgesia administration. Morphine patient controlled analgesia (PCA) has been demonstrated to be safe and effective in the burn-injured population [149]. Because it adds to the patient's sense of control and decreases the length of time needed to medicate a patient, PCA is the mode of choice at many burn centers. At times that the patient is unable to operate the PCA device, it may be nurse activated for painful procedures and breakthrough pain. A typical dose of morphine administered for procedural pain is 5 mg to 10 mg, but the dose will vary depending on the patient's requirements [130].

For patients taking oral analgesics, hydromorphone, morphine, or oxycodone is recommended. Yet, fentanyl can be given IV or through the transmucosal route of administration (e.g., fentanyl oralet) [131]. Patients should be observed to insure proper administration (i.e., oralets should not be chewed and swallowed, although some patients may be tempted to do so). For outpatients, short-acting oral opioids, such as oxycodone, are usually effective.

Recently, intranasal fentanyl was evaluated in a study of patients (n=26) undergoing burn dressing changes, and was found to be similar in efficacy and safety to

oral morphine [150]. For patients who do not tolerate oral analgesic administration and for whom IV access is not available, the intranasal route may be an effective alternative.

In a pilot study (n=5), alfentanil PCA was found to relieve pain during dressing changes in patients during the acute phase of burn recovery [151]. Further research is needed to evaluate its pharmacokinetics and efficacy in this patient population.

Another study evaluated the effectiveness of fentanyl PCA for procedural burn pain [152]. In a randomized, double-blinded study of patients with ≥20% TBSA burns (n=60), a loading dose of fentanyl (1 μg/kg) was given and patients' pain intensity scores were monitored using a 0 to 10 visual analogue scale (VAS). Whenever their VAS was >2, patients were permitted to self administer fentanyl in a bolus dose of 10 μg, 20 μg, 30 μg or 40 μg with a 5 minute lockout. Patients who received bolus doses in the 10 μg and 20 μg range had significantly (all $P<0.0001$) higher mean VAS scores (7.3 ± 1.33 and 7.20 ± 1.21, respectively) than those who received doses in the 30 μg and 40 μg range (4.47 ± -0.83 and 3.90 ± 0.63, respectively). No hemodynamic instability or respiratory depression was noted among the participants. Findings from this study suggest that fentanyl PCA can be a safe and effective method of analgesia for procedural pain in burn-injured patients.

A cautionary statement about the use of fentanyl is warranted. Fentanyl has the advantages of high potency and rapid onset. However, at steady-state, slow elimination from tissues can lead to a prolonged half life ($t_{1/2}$ = ~12 hours) [153]. Thus, it is important not to confuse the fast acting properties of fentanyl with its potential for a long half life, which may contribute to the risk of sedation and/or undesirable side effects over time of administration.

Intravenous ketamine has been used successfully for years for the treatment of burn procedural pain in children [154]. Recently, ketamine was introduced in an elixir for oral administration. A study that evaluated its effectiveness in pediatric patients reported positive results (n=19, 400% reduction in pain, $P< 0.05$) [155]. However, oral ketamine was not effective in a study of adult volunteers undergoing experimental burn injury (n=24) [156].

At sub-anesthetic doses, ketamine may be used in combination with an opioid, such as morphine, to control procedural and background pain. Because opioids can be used more sparingly when combined with ketamine, the adverse effects of opioid therapy can be reduced. Furthermore, because ketamine is an NMDA receptor antagonist, it has the potential to affect the symptoms associated with central sensitization (i.e., secondary hyperalgesia, allodynia, temporal summation and neuropathic pain). This theory was tested in a study of volunteers who underwent experimental burn injury (n=11) [157]. The combination of IV ketamine and morphine abolished symptoms of temporal summation, whereas the administration of either ketamine or morphine alone did not. While the study was conducted in healthy volunteers under experimental conditions, these results warrant further investigation. Another benefit of the use of ketamine is that it has an amnestic effect at sub-anesthetic doses. A limitation of the use of ketamine is that its administration requires specialized expertise and careful monitoring which may limit the practicality of its use in some settings. Ketamine may induce emergence hallucinations, which are often minimized with the concomitant use of a benzodiazepine.

Regional nerve blocks are effective for postoperative pain relief and have been used successfully in the burn-injured patient. However, an acute pain service is usually

required to provide the expertise and oversight necessary for this mode of analgesia. Moreover, continuous access (i.e., through an insertion site) is often contraindicated due to the risk of infection in this immunosuppressed patient population. In addition, daily dressing changes and topical antimicrobials introduce moisture that can penetrate and dislodge dressings increasing the risk of infection. Thus, continuous nerve block insertion sites must be monitored closely. Epidural infusions can be administered caudally, particularly in children under 6 months of age, or through lumbar or thoracic epidural catheters in older children and adults [134].

The use of catheters for continuous nerve blocks has been established in burned children, although in most cases only one catheter is used. A case was reported of managing pain in a burn-injured 3-year-old child who underwent a toe-to-finger transfer with 2 regional catheters: axillary and sciatic [158]. A pain score of 0 was noted during the entire study period. The total dose of bupivacaine was limited to an acceptable range, and the child recovered completely. This report adds to growing evidence in favor of the safety and efficacy of continuous peripheral nerve blocks in pediatric patients. These authors concluded that double continuous nerve blocks allow optimal analgesia in burned children after complex orthopedic surgery without major adverse events. In addition, plasma concentrations of bupivacaine remained small during the study period.

A common clinical observation in patients experiencing postoperative pain following a skin grafting procedure is that the split-skin donor site is substantially more painful than the grafted site. To address this problem, a prospective, randomized, double-blind study assessed the efficacy of a continuous fascia iliac compartment block (FICB) at the thigh donor site (n=20) [159]. Patients with continuous FICB had significantly

reduced postoperative morphine consumption than control patients at all time points over 72 hours (23 ± 20mg versus 88 ± 29mg, respectively; $P < 0.05$). In both groups, VAS scores remained low but were only significantly lower for patients with continuous FICB during the first dressing change ($P < 0.05$). This study suggests that continuous FICB is an effective method for managing pain at the thigh donor site in patients with burns following skin grafting procedures.

A prolonged nerve block was administered before an experimental partial-thickness burn injury (n=20) to determine if it could reduce local inflammation and late hyperalgesia after recovery from the block [160]. A total of six subjects were excluded because of insufficient initial block (n=2) or because the block lasted beyond the study period (n=4). The remaining 14 subjects experienced significantly reduced primary ($P=0.005$) and secondary hyperalgesia ($P=0.01$) in the blocked leg compared to the unblocked leg. Erythema intensity and blister formation were not significantly affected by the blockade ($P=0.94$ and $P=0.07$, respectively). These data suggest that a prolonged, preemptive nerve block reduced late hyperalgesia after thermal burn, whereas the erythema and blister formation were not significantly affected.

Clinically, topical anesthetics such as EMLA® (eutectic mixture of local anesthetics, 2.5% lidocaine and 2.5% prilocaine) cream have not been successful in alleviating burn pain. These results are disappointing and appear to be due to poor tissue penetration [161]. Burn care providers have also used topical local anesthetics in special cases with caution due to the risk of cardiac arrhythmias associated with systemic absorption [162,163]. However, in a study of patients undergoing skin graft procedures (n=60), an aerosol spray of lidocaine 2% was found to be safe and effective when applied

to donor sites intraoperatively [164]. Pain scores and 24-hour opioid use were lower in the group who received lidocaine 2% compared to the groups who received topical bupivacaine 0.5% or placebo. Local anesthetic solutions aerosolized onto skin-harvest sites did not affect healing or produce toxic blood concentrations.

The analgesic effect of topically applied ketamine [165] and the nonsteroidal antiinflammatory drug (NSAID) ketorolac [166] have also been evaluated in the burn-injured patient population without success. In addition, morphine has been applied topically (e.g., infused with the antimicrobial cream sulfadiazine), resulting in a slightly more analgesic effect in the experimental group over placebo, but the difference was not statistically significant [167]. Subcutaneous local administration of morphine was effective in attenuating pain following an experimental thermal injury [168]. However, subcutaneous injection of burn injuries is not practical due to the wide area they usually involve, nor would it likely be well tolerated in the clinical setting.

Clinical Management of Procedural Anxiety During the Acute Phase

In addition to pain, anxiety may rapidly increase during the acute phase of burn recovery. In a study of hospitalized burn-injured patients (n=24), procedural pain and anxiety scores increased over the first 4 days of hospitalization and pain was highly correlated with anxiety [169]. For anxiety associated with procedural pain, benzodiazepines such as IV midazolam and lorazepam have been effective in adults and children with burns [130,131,170,171]. Other routes of administration have also been effective, particularly in children, including the intranasal [172,173] and rectal [174] routes.

Clinical Management of Background Pain During the Acute Phase

Intravenous opioids (e.g., fentanyl or morphine) are recommended during the acute phase for those patients with IV access [131]. Background pain can be brought under control by starting with loading doses, and then establishing a dose for continuous infusion [121]. A continuous infusion should be considered during those times that the patient is experiencing severe pain, but is not able to maintain analgesia by PCA (e.g., due to limited dexterity associated with their injuries, during planned sleep intervals, or when the patient is deeply sedated). Doses can be titrated based on pain intensity ratings and safety parameters.

Oral medications that are effective in the treatment of background pain during the acute phase include controlled release morphine sulfate or oxycodone or immediate release methadone [131]. Scheduled doses of oral codeine and acetaminophen or ibuprofen may also be utilized but are less effective.

Clinical Management of Breakthrough Pain During the Acute Phase

If IV access is an option, a bolus dose of opioid is established for PCA administration by the patient as needed. A typical dose of morphine for breakthrough pain is 2 mg every 10 minutes, with a 30 mg 4-hour lockout (i.e., limit set) [130]. Rapid onset opioids such as transmucosal fentanyl citrate (i.e., fentanyl oralet) or oral hydromorphone may also be utilized.

The Healing Phase

Pain can significantly compromise recovery during the healing phase. Thus, there is often an increased need for large doses of systemic analgesic agents during this phase. The primary goals during the healing phase are to manage the patient's pain while, at the

same time, promoting functional independence, nutritional and fluid intake, and participation in therapies.

The type of pain management during the healing phase often depends on the anticipated length of stay. This is because, prior to discharge, patients are converted from IV to oral opioids to promote independence and participation in their plan of care. It is recommended that opioids be given in a controlled-release preparation on an around-the-clock, scheduled basis. Scheduled dosing is preferred because it provides steady state serum concentrations and avoids 'peaks' and 'troughs' that can hamper analgesic therapy [134,144]. It should be noted that non-opioid analgesics and nonpharmacologic interventions are very useful adjuncts, in addition to opioids, as methods to promote analgesia following burn injury [8,175], including music [176-178], hypnosis [179-181], and virtual reality [182,183] (For review [8]).

Clinical Management of Procedural Pain During the Healing Phase

Inhaling nitrous oxide is an effective adjunct to morphine for dressing changes for some patients. Patients may self-administer the gas mixed with oxygen. Nitrous oxide is recommended for use with children [184,185], but contraindicated during pregnancy [186] and in the presence of disease or traumatic injury that affects the head or chest [187,188].

For outpatients undergoing painful procedures, oral transmucosal fentanyl citrate (i.e., fentanyl oralet) is a rapid-acting analgesic that is accepted readily by children and adults alike. Transmucosal fentanyl 10 μg/kg was as safe and effective as oral oxycodone 0.2 mg/kg in children 5 to 14 years of age (n=22) undergoing burn dressing change procedures in the outpatient setting [189].

Clinical Management of Background Pain During the Healing Phase

If the patient's pain is not well controlled with oral analgesics, IV administration should be resumed. The conversion ratio for IV to oral opioids will depend on the individual patient. However, a goal of pain management during the healing phase is to control pain without interfering with the patient's ability to perform activities of daily living, including fluid and nutritional support, and rehabilitation therapies such as range of motion exercises.

Clinical Management of Breakthrough Pain During the Healing Phase

Breakthrough pain is expected to occur during the healing phase, which means it can be anticipated and planned for in advance. It is precipitated by a voluntary action, such as movement and has also been referred to as incident pain [153]. Breakthrough pain during the healing phase can be treated effectively with a 'rescue' dose of an immediate-release opioid. The same opioid analgesic and route of administration recommended for background pain management may also be used to manage breakthrough pain. For example, if a patient is taking controlled-release morphine for background pain, it is recommended that immediate-release morphine be given for breakthrough pain [153]. If the patient is taking methadone, a short-half life opioid such as fentanyl or morphine is recommended [190] to provide rescue doses when breakthrough pain occurs.

The Rehabilitation Phase

Ideally, upon entering the rehabilitation phase the patient has usually been weaned from intravenous morphine to an oral opioid analgesic for the management of procedural pain. Short-acting opioids that are combined with acetaminophen, such as oxycodone are

usually effective during this phase. However, the management of larger and more complex burns on an outpatient basis may require that patients be discharged on controlled-release opioids for background pain as well. Thus, many of the recommendations for inpatient pain management may also be suitable for use at home.

During the rehabilitation phase, complaints of neuropathic pain may first arise. Some co-analgesics have been successfully used to treat neuropathic pain following burn injury, including antidepressants such as amytriptyline and fluoxetine; and anticonvulsants, such as gabapentin [191]. In addition, systemic administration of lidocaine by IV infusion has been reported to be effective for both acute [192] and neuropathic pain following burn injury (For review [193]). However, more research is needed to establish safety and efficacy parameters for this treatment.

Clinical Management of Procedural Pain During the Rehabilitation Phase

For severe pain, hydropmorphone may be used. For mild to moderate pain, oxycodone, NSAIDs or a combination of the two may be effective. Non-pharmacologic interventions such as massage therapy have been reported to be particularly useful for patients during the rehabilitation phase of burn recovery when opioids are administered less frequently [194].

Clinical Management of Background Pain During the Rehabilitation Phase

During the healing phase, wound closure has been accomplished or is close to being accomplished. Thus, background pain is usually not a problem during the rehabilitation phase. If it is, NSAIDs are excellent analgesic agents for background pain during the rehabilitation phase due to their anti-inflammatory properties. However, recent evidence of increased risk of cardiovascular events associated with prolonged use of

certain NSAIDs suggests that these medications should be recommended judiciously until the full ramifications of their use are understood [195].

Of note, intense itching intermittently takes the place of pain during this phase. Itching is thought to be exacerbated by inflammatory mediators released during angiogenesis and nerve regeneration, but further research is needed to study this problem. Antihistamines may attenuate itching, but the need exists to find more effective therapies for this major source of suffering during the rehabilitation phase of burn recovery.

Clinical Management of Breakthrough Pain During the Rehabilitation Phase

During the rehabilitation phase, physical and occupational therapy are critically needed to achieve optimal functional recovery. Thus, pain associated with range of motion exercises may be an issue. Short-acting opioids such as oxycodone and morphine may be used for breakthrough pain during the rehabilitation phase.

Summary

Pain management of the burn-injured patient is very complex due to its severity and variability, often increasing in intensity over time. Moreover, management of burn pain requires the assessment and management of three different types of pain over three phases of recovery. The effective management of pain associated with burn injury is possibly the most challenging aspect of burn care.

References

1. Marvin, J.A., et al., *Burn nursing Delphi study. Setting research priorities.* J Burn Care Rehabil, 1991. **12**(2): p. 190-7.
2. Choinière, M., *Burn pain: A unique challenge.* Pain--Clinical Updates, 2001(Spring).
3. Choinière, M., Melzack, R., Girard, N., Rondeau, J., Paquin, M. J., *Comparisons between patients' and nurses' ratings of pain and medication efficacy in severe burn injuries.* Pain, 1990. **40**: p. 143-152.
4. Gilboa, D., Friedman, M, & Tsur, H, *The burn as a continuous traumatic stress: Implications for emotional treatment during hospitalization.* Journal of Burn Care and Rehabilitation, 1994. **15**: p. 86-91.
5. Iafrati, N.S., *Pain on the burn unit: patient vs nurse perceptions.* J Burn Care Rehabil, 1986. **7**(5): p. 413-6.
6. Taal, L.A. and A.W. Faber, *Burn injuries, pain and distress: exploring the role of stress symptomatology.* Burns, 1997. **23**(4): p. 288-90.
7. Ulmer, J.F., *An exploratory study of pain, coping, and depressed mood following burn injury.* J Pain Symptom Manage, 1997. **13**(3): p. 148-57.
8. Patterson, D.R., et al., *Optimizing control of pain from severe burns: a literature review.* Am J Clin Hypn, 2004. **47**(1): p. 43-54.
9. Honari, S., et al., *Comparison of pain control medication in three age groups of elderly patients.* J Burn Care Rehabil, 1997. **18**(6): p. 500-4.
10. Montgomery, R.K., *Pain management in burn injury.* Crit Care Nurs Clin North Am, 2004. **16**(1): p. 39-49.

11. Latarjet, J. and M. Choinière, *Pain in burn patients.* Burns, 1995. **21**(5): p. 344-8.

12. Carr, D.B., et al., eds. *Acute Pain Management: Operative or Medical Procedures and Trauma.* Clinical Practice Guideline No. 1, ed. A.P.M.G. Panel. Vol. No. 1. 1992, Agency for Health Care Policy and Research, U.S. Department of Health and Human Services, Public Health Service: Rockville, MD.

13. Max, M.B., et al., *Principles of Analgesic Use in the Treatment of Acute Pain and Cancer Pain.* 4th ed. 1999, Glenview, IL: American Pain Society. 1-64.

14. Ulmer, J.F., *Burn pain management: a guideline-based approach.* J Burn Care Rehabil, 1998. **19**(2): p. 151-9.

15. Ready, L.B. and W.T. Edwards, eds. *Management of Acute Pain: A Practical Guide.* IASP Publications, ed. T.F.o.A. Pain. 1992, International Association for the Study of Pain (IASP): Seattle.

16. Choinière, M., Melzack, R, Rondeau, J., et al., *The pain of burns: Characteristics and correlates.* Journal of Trauma, 1989. **29**: p. 1531-1539.

17. Jonsson, C.E., et al., *Background pain in burn patients: routine measurement and recording of pain intensity in a burn unit.* Burns, 1998. **24**(5): p. 448-54.

18. Ptacek, J.T., D.R. Patterson, and J. Doctor, *Describing and predicting the nature of procedural pain after thermal injuries: implications for research.* J Burn Care Rehabil, 2000. **21**(4): p. 318-26.

19. Silbert, B.S., Osgood, P F, Carr, D B, *Burn pain*, in *Anesthesia: Biologic Foundations*, T. Yaksh, Lynch III, C, Zapol, W M, Maze, M, Biebuyck, J F, Saidman, L J, Editor. 1999, Lippincott-Raven: Philadelphia.

20. Brigham, P.A., *American Burn Association Fact Sheet: Burn incidence and treatment in the United States.* 2000: Chicago, IL.

21. Singer, A.J. and H.C. Thode, Jr., *National analgesia prescribing patterns in emergency department patients with burns.* J Burn Care Rehabil, 2002. **23**(6): p. 361-5.

22. National Hospital Discharge Survey, *Hospital admissions.* 1995 to 1998.

23. Agency for Health Care Policy and Research, *HCUP-II data.* 1990 to 1993.

24. DRG Handbook. 1996.

25. American Burn Association, *Burn Center Admissions.* 1991.

26. National Hospital Ambulatory Medical Care Survey, *Emergency Department Visits.* 1992 to 1995.

27. National Medical Expenditure Survey, *Injuries represented in Emergency Department Visits.* 1987.

28. Hunt, J.L. and G.F. Purdue, *Prevention of burn injuries*, in *Total Burn Care*, D. Herndon, Editor. 2002, W. B. Saunders: New York. p. 33-39.

29. American Burn Association, *Burn Patient Registry.* 1991 to 1993.

30. National Burn Information Exchange. 1965 to 1985.

31. Runyon, C.W., et al., *Risk factors for fatal residential fires.* New England Journal of Medicine, 1992. **327**: p. 859-863.

32. Graitcer, P.L. and J.E. Sniezek, *Hospitalization due to tap water scalds.* MMWR, 1978-1985. **37**: p. 35-38.

33. Blackmore, J., *Ludwig Boltzmann, His Later Life and Philosophy, 1900-1906 Book One: A documentary history.* 1995, Boston: Kluwer Academic Publishers.

34. Lee, R.C.A., R. D., *The physiochemical basis for thermal and non-thermal 'burn' injuries.* Burns, 1996. **22**(7): p. 509-519.

35. Gershfeld, N.L. and M. Murayama, *Thermal instability of red blood cell membrane bilayers: Temperature dependence of hemolysis.* Journal of Membrane Biology, 1968. **101**: p. 62-72.

36. Moritz, A.R. and F.C. Henriques, *Studies of thermal injury, II. The relative importance of time and surface temperature in the causation of cutaneous burns. An experimental study.* American Journal of Pathology, 1947. **23**: p. 695-720.

37. Lawrence, C., Atac, B, *Hematologic changes in massive burn injury.* Critical Care Medicine, 1992. **20**: p. 1284-1288.

38. LaMotte, R.H. and J.N. Campbell, *Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgments of thermal pain.* Journal of Neurophysiology, 1978. **41**: p. 509-528.

39. Tillman, D.B., et al., *Response of C fibre nociceptors in the anesthetized monkey to heat stimuli: Correlation with pain threshold in humans.* Journal of Physiology, 1995. **485**((Pt 3)): p. 767-774.

40. Pruitt, B.A., Goodwin, C W, Mason Jr, A D, *Epidemiological, demographic, and outcome characteristics of burn injury*, in *Total Burn Care*, D. Herndon, Editor. 2002, W B Saunders: New York.

41. Stucky, C.L., Gold, M. S., Zhang, X., *Mechanisms of pain.* Proceedings of the National Academy of Sciences, 2001. **98**(21): p. 11845-11846.

42. Heimbach, D.M., *Burn Pearls.* 15 ed. 1996, Seattle, WA: University of Washington Burn Center at Harborview. 1-69.

43. Carrougher, G., ed. *Burn Care and Therapy.* 1998, Mosby, Inc.: St. Louis.

44. Demling, R.H.D., L., *Partial-Thickness Burns: Current concepts as to pathogenesis and treatment; the role for a bioengineered skin substitute.* 1999, Advanced Tissue Sciences, Inc. p. 1-89.

45. Meyer, W.J., et al., *Management of pain and other discomforts in burned patients,* in *Total Burn Care,* D. Herndon, Editor. 2002, W. B. Saunders: Philadelphia. p. 747-765.

46. Jackson, D.M., *In search of an acceptable burn classification.* British Journal of Plastic Surgery, 1970. **23**: p. 219-226.

47. Moritz, A.R. and F.C. Henriques, *Studies of thermal injuyr, III. The pathology and pathogenesis of cutaneous burns. An experimental study.* American Journal of Pathology, 1947. **23**: p. 915-940.

48. Deitch, E.A., *A policy of early excision and grafting in elderly burn patients shortens the hospital stay and improves survival.* Burns, 1985. **12**: p. 109-114.

49. Herndon, D.N., et al., *A comparison of conservative versus early excision therapies in severely burned patients.* Annals of Surgery, 1989. **209**: p. 547-553.

50. Munster, A.M., M. Smith-Meek, and P. Sharkey, *The effect of early surgical intervention on mortality and cost-effectiveness in burn care.* Burns, 1994. **20**(1): p. 61-64.

51. Kowalske, K. and D.L. Tanelian, *Burn pain evaluation.* Anesthesiology Clinics of North America, 1997. **15**: p. 269-283.

52. Meyer, R.A. and J.N. Campbell, *Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand.* Science, 1981. **213**(4515): p. 1527-9.

53. Monafo, W.W., *Physiology of pain.* Journal of Burn Care and Rehabilitation, 1995. **16**: p. 345-347.

54. Arturson, G., *Pathophysiology of the burn wound and pharmacological treatment. The Rudi Hermans Lecture, 1995.* Burns, 1996. **22**(4): p. 255-274.

55. Löfgren, O., Y. Qi, and T. Lundeberg, *Inhibitory effects of tachykinin receptor antagonists on thermally induced inflammatory reactions in a rat model.* Burns, 1999. **25**(2): p. 125-9.

56. Imaizumi, T., et al., *Interleukin-1 levels in blister fluids of some skin diseases with blister formation.* Clin Physiol Biochem, 1990. **8**(4): p. 179-83.

57. Ono, I., et al., *A study of cytokines in burn blister fluid related to wound healing.* Burns, 1995. **21**(5): p. 352-355.

58. Rose, M., et al., *Factors altering the sleep of burned children.* Sleep, 2001. **24**(1): p. 45-51.

59. Kowal-Vern, A., Sharp-Pucci, M. M., Walenga, J. M., Dries, D. J., Gamelli, R. L., *Trauma and thermal injury: Comparison of hemostatic and cytokine changes in the acute phase of injury.* Journal of Trauma, 1998. **44**(2): p. 325-329.

60. Dunnick, C.A., N.S. Gibran, and D.M. Heimbach, *Substance P has a role in neurogenic mediation of human burn wound healing.* Journal of Burn Care and Rehabilitation, 1996. **17**(5): p. 390-6.

61. Onuoha, G.N. and E.K. Alpar, *Levels of vasodilators (SP, CGRP) and vasoconstrictor (NPY) peptides in early human burns.* Eur J Clin Invest, 2001. **31**(3): p. 253-7.

62. Gibran, N.S., Heimbach, D M, *Mediators in thermal injury. Seminars in Nephrology.* Seminars in Nephrology, 1993. **13**(4): p. 344-358.

63. Feezor, R.J., et al., *Temporal patterns of gene expression in murine cutaneous burn wound healing.* Physiological Genomics, 2004. **16**: p. 341-348.

64. Shakespeare, P., *Burn wound healing and skin substitutes.* Burns, 2001. **27**: p. 517-522.

65. Linares, H.A., *From wound to scar.* Burns, 1996. **22**: p. 339-352.

66. Beales, J.G., *Factors influencing the expectation of pain among patients in a children's burns unit.* Burns, 1983. **9**(3): p. 187-92.

67. Savedra, M., *Coping with pain: strategies of severely burned children.* Can Nurse, 1977. **73**(8): p. 28-9.

68. Nwariaku, F.E., et al., *Effect of bradykinin antagonist on the local inflammatory response following thermal injury.* Burns, 1996. **22**(4): p. 324-327.

69. Dobke, M.K., E.C. Hayes, and C.R. Baxter, *Leukotrienes LTB4 and LTC4 in thermally injured patients' plasma and burn blister fluid.* Journal of Burn Care and Rehabilitation, 1987. **8**(3): p. 189-91.

70. Kowal-Vern, A., Walenga, J. M., Sharp-Pucci, M., Hoppensteadt, D., Gamelli, R. L., *Postburn edema and related changes in interleukin-2, leukocytes, platelet activation, endothelin-1, and C1 esterase inhibitor.* Journal of Burn Care and Rehabilitation, 1997. **18**(2): p. 99-103.

71. Shimizu, S., Tanaka, H., Sakaki, S., Yukioka, T., Matsuda, H. et al., *Burn depth affects dermal interstitial fluid pressure, free radical production, and serum histamine levels in rats.* The Journal of Trauma, 2002. **52**(4): p. 683-687.

72. Kessler, W., et al., *Excitation of cutaneous afferent nerve endings in vitro by a combination of inflammatory mediators and conditioning effect of substance P.* Experimental Brain Research, 1992. **91**: p. 467-476.

73. Nicol, G.D. and M. Cui, *Enhancement by prostaglandin E_2 of bradykinin activation of embryonic rat sensory neurones.* Journal of Physiology (London), 1994. **480**: p. 485-492.

74. Stucky, C.L., Thayer, S. A., & Seybold, V. S., *Prostaglandin E_2 increases the proportion of neonatal rat dorsal root ganglion neurons that respond to bradykinin.* Neuroscience, 1996. **74**: p. 1111-1123.

75. Hong, Y. and F.V. Abbott, *Behavioural effects of intraplantar injection of inflammatory mediators in the rat.* Neuroscience, 1994. **63**: p. 827-836.

76. Abbott, F.V., Y. Hong, and P. Blier, *Activation of 5-HT2A receptors potentiates pain produced by inflammatory mediators.* Neuropharmacology, 1996. **35**: p. 99-110.

77. Stucky, C.L., L.G. Abrahams, and V.S. Seybold, *Bradykinin increases the proportion of neonatal rat dorsal root ganglion neurons that respond to capsaicin and protons.* Neuroscience, 1998. **84**: p. 1257-1265.

78. Kress, M., P.W. Reeh, and L. Vyklicky, *An interaction of inflammatory mediators and protons in small diameter dorsal root ganglion neurons of the rat.* Neuroscience Letters, 1997. **224**: p. 37-40.

79. Li, C., R.W. Peoples, and F.F. Weight, *Acid pH augments excitatory action of ATP on a dissociated mammalian sensory neuron.* Neuroreport, 1996. **7**: p. 2151-2154.

80. Steen, K.H., Steen, A. E., Kreysel, H. W., & Reeh, P. W., *Inflammatory mediators potentiate pain induced by experimental tissue acidosis.* Pain, 1996. **66**: p. 163-170.

81. Alessandri-Haber, N., et al., *Transient receptor potential vanilloid 4 is essential in chemotherapy-induced neuropathic pain in the rat.* Journal of Neuroscience, 2004. **24**(18): p. 4444-52.

82. Alessandri-Haber, N., et al., *Hypotonicity induces TRPV4-mediated nociception in rat.* Neuron, 2003. **39**(3): p. 497-511.

83. Guler, A.D., et al., *Heat-evoked activation of the ion channel, TRPV4.* J Neurosci, 2002. **22**(15): p. 6408-14.

84. Vriens, J., et al., *Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4.* Proceedings of the National Academy of Sciences, 2004. **101**(1): p. 396-401.

85. Lewis, T., *Experiments relating to cutaneous hyperalgesia and its spread through somatic fibres.* Clinical Science, 1935. **2**: p. 373-423.

86. Lewis, T., *Pain.* 1942, New York: Macmillan.

87. Basbaum, A.I., *Basic Mechanisms*, in *Pain Management Secrets*, R. Kanner, Editor. 2003, Hanley & Belfus, Inc.: Philadelphia. p. 10-15.

88. Youn, Y.K., C. LaLonde, and R. Demling, *The role of mediators in the response to thermal injury.* World J Surg, 1992. **16**(1): p. 30-6.

89. Rodriguez, J.L., et al., *Correlation of the local and systemic cytokine response with clinical outcome following thermal injury.* J Trauma, 1993. **34**(5): p. 684-94; discussion 694-5.

90. Ward, P.A. and G.O. Till, *Pathophysiologic events related to thermal injury of skin.* J Trauma, 1990. **30**(12 Suppl): p. S75-9.

91. Jonsson, C.E., E. Granstrom, and M. Hamberg, *Prostaglandins and thromboxanes in burn injury in man.* Scand J Plast Reconstr Surg, 1979. **13**(1): p. 45-7.

92. Nwariaku, F., et al., *Inhibition of selectin- and integrin-mediated inflammatory response after burn injury.* Journal of Surgical Research, 1996. **63**(1): p. 355-358.

93. Gamelli, R.L., et al., *Burn-induced nitric oxide release in humans.* Journal of Trauma, 1995. **39**(5): p. 869-77; discussion 877-8.

94. McCarson, K.E. and J.E. Krause, *NK-1 and NK-3 type tachykinin receptor mRNA expression in the rat spinal cord dorsal horn is increased during adjuvant or formalin-induced nociception.* J Neurosci, 1994. **14**(2): p. 712-20.

95. Traub, R.J., *The spinal contribution of substance P to the generation and maintenance of inflammatory hyperalgesia in the rat.* Pain, 1996. **67**(1): p. 151-61.

96. Woolf, C.J., *Recent advances in the pathophysiology of acute pain.* British Journal of Anaesthesia, 1989. **63**: p. 139-146.

97. Woolf, C.J., *Evidence for a central component of postinjury pain hypersensitivity.* Nature, 1983. **308**: p. 686-688.

98. Wall, P.D. and C.J. Woolf, *Muscle but no cutaneous C-afferent input produces prolonged increase in the excitability of the flexion reflex in the rat.* Journal of Physiology London, 1984. **356**: p. 443-458.

99. Cook, A.J., et al., *Dynamic receptive field plasticity in rat spinal cord dorsal horn following C-primary afferent input.* Nature, 1987. **325**(7000): p. 151-3.

100. Raja, S.N., J.N. Campbell, and R.A. Meyer, *Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin.* Brain, 1984. **107 (Pt 4)**: p. 1179-88.

101. LaMotte, R.H., et al., *Neurogenic hyperalgesia: Psychophysical studies of underlying mechanisms.* Journal of Neurophysiology, 1991. **66**: p. 190-211.

102. Torebjörk, H.E., J.M. Lundberg, and R.H. LaMotte, *Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans.* Journal of Physiology, 1992. **448**: p. 765-780.

103. Warncke, T., A. Stubhaug, and E. Jorum, *Ketamine, an NMDA receptor antagonist, suppresses spatial and temporal properties of burn-induced secondary hyperalgesia in man: a double-blind, cross-over comparison with morphine and placebo.* Pain, 1997. **72**(1-2): p. 99-106.

104. Warncke, T., A. Stubhaug, and E. Jorum, *Preinjury treatment with morphine or ketamine inhibits the development of experimentally induced secondary hyperalgesia in man.* Pain, 2000. **86**(3): p. 293-303.

105. Ilkjaer, S., et al., *Effect of systemic N-methyl-D-aspartate receptor antagonist (dextromethorphan) on primary and secondary hyperalgesia in humans.* Br J Anaesth, 1997. **79**(5): p. 600-5.

106. Price, D.D., et al., *Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses.* Pain, 1977. **3**: p. 57-68.

107. Adriaensen, H., et al., *Nociceptor discharges and sensations due to prolonged noxious mechanical stimulation--a paradox.* Human Neurobiology, 1984. **3**: p. 53-58.

108. Torebjörk, H.E., *Nociceptor activation and pain.* Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 1985. **308**: p. 227-304.

109. Koltzenburg, M. and H.O. Handwerker, *Differential ability of human cutaneous nociceptors to signal mechanical pain and to produce vasodilation.* Journal of Neuroscience, 1994. **14**: p. 1756-1765.

110. Pedersen, J.L., et al., *Hyperalgesia and temporal summation of pain after heat injury in man.* Pain, 1998. **74**(2-3): p. 189-97.

111. Ham, A.W., *Experimental study of the histopathology of burns: With particular reference to sites of fluid loss in burns of different depths.* Annals of Surgery, 1944. **120**: p. 689-697.

112. de Camara, D.L., et al., *Progression of thermal injury: A morphologic study.* Plastic Reconstructive Surgery, 1982. **69**(491-499).

113. Hallin, R.G., Wu, G., *Fitting the pieces in the peripheral nerve puzzle.* Experimental Neurology, 2001. **172**: p. 482-492.

114. Zimmermann, M., *Pathobiology of neuropathic pain.* Eur J Pharmacol, 2001. **429**(1-3): p. 23-37.

115. Stedman, T.L., *Stedman's Medical Dictionary*. 27th ed, ed. M.B. Pugh. 2000, Philadelphia: Lippincott Williams & Wilkins.

116. Carlton, S.M., G.L. Hargett, and R.E. Coggeshall, *Plasticity in alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunits in the rat dorsal horn following deafferentation.* Neuroscience Letters, 1998. **242**(1): p. 21-4.

117. Marvin, J., *Management of pain and anxiety*, in *Burn Care and Therapy*, G.J. Carrougher, Editor. 1998, Mosby: St. Louis. p. 167-183.

118. Watkins, P.N., et al., *The role of the psychiatrist in the team treatment of the adult burn patient with burns.* Journal of Burn Care and Rehabilitation, 1992. **13**: p. 19-27.

119. Ashburn, M.A., *Burn pain: The management of procedure-related pain.* Journal of Burn Care and Rehabilitation, 1995. **16**(3, Part 2): p. 365-371.

120. Morse, J.M. and C. Mitcham, *The experience of agonizing pain and signals of disembodiment.* J Psychosom Res, 1998. **44**(6): p. 667-80.

121. Lauterbach, S. and R. George, *Burn Pain*, in *Pain: Clinical Manual*, M.M.C. Pasero, Editor. 1999, Mosby: St. Louis. p. 527-531.

122. Atchison, N.E., et al., *Pain during dressing change in children: Relationship to burn area, depth and analgesic regimens.* Pain, 1991. **47**: p. 41-45.

123. Perry, S., G. Heidrich, and E. Ramos, *Assessment of pain in burned patients.* Journal of Burn Care and Rehabilitation, 1981. **2**: p. 322-326.

124. Schreiber, S. and T. Galai-Gat, *Uncontrolled pain following physical injury as the core-trauma in post-traumatic stress disorder.* Pain, 1993. **54**(1): p. 107-10.

125. Ptacek, J.T., et al. *The relationship between pain during hospitalization and disability at one month post burn.* in *Annual Meeting of the American Burn Association.* 1994. Orlando, Florida.

126. Taal, L.A. and A.W. Faber, *Post-traumatic stress, pain and anxiety in adult burn victims.* Burns, 1997. **23**(7-8): p. 545-9.

127. Difede, J., et al., *Determinants of pain expression in hospitalized burn patients.* Pain, 1997. **72**(1-2): p. 245-51.

128. Bauer, K.M., P.E. Hardy, and B. Van Dorsten, *Posttraumatic stress disorder in burn populations: A critical review of the literature.* Journal of Burn Care and Rehabilitation, 1998. **19**: p. 230-240.

129. Perry, S., D.F. Cella, and J. Falkenberg, *Pain perception in burn patients with stress disorder.* Journal of Pain and Symptom Management, 1987. **2**: p. 29-33.

130. Parkland Health & Hospital System, D., *Parkland Burn Acute Care Unit Adult Pain Management Pathway*, in *Pain: Clinical Manual*, M.M.C. Pasero, Editor. 1999, Mosby: St. Louis. p. 529.

131. Carrougher, G.J., et al., *Comparison of patient satisfaction and self-reports of pain in adult burn-injured patients.* Journal of Burn Care and Rehabilitation, 2003. **24**(1): p. 1-8.

132. Williams, P.I., R.E. Sarginson, and J.M. Ratcliffe, *Use of methadone in the morphine-tolerant burned paediatric patient.* Br J Anaesth, 1998. **80**(1): p. 92-5.

133. Portenoy, R.K. and N.A. Hagen, *Breakthrough pain: Definition, prevalence and characteristics.* Pain, 1990. **41**: p. 273-281.

134. American Pain Society, *Principles of Analgesic Use in the Treatment of Acute Pain and Cancer Pain.* 5th ed. 2003, Glenview, IL: American Pain Society, A National Chapter of the International Association for the Study of Pain. 1-73.

135. Heimbach, D., *Foreward,* in *Burn Care and Therapy,* R.N. Gretchen J. Carrougher, M.N., Editor. 1998, Mosby, Inc.: St. Louis. p. ix-x.

136. Heimbach, D., Engrav, L, Grube, B, et al., *Burn depth: A review.* World Journal of Surgery, 1992. **16**: p. 10-15.

137. Choinière, M., Melzack, R., Papillon, J., *Pain and paresthesia in patients with healed burns: An exploratory study.* Journal of Pain and Symptom Management, 1991. **6**(7): p. 437-444.

138. Malenfant, A., et al., *Prevalence and characteristics of chronic sensory problems in burn patients.* Pain, 1996. **67**(2-3): p. 493-500.

139. Stella, M., et al., *Sensory endings in skin grafts and scars after extensive burns.* Burns, 1994. **20**(6): p. 491-5.

140. Malenfant, A., et al., *Tactile, thermal and pain sensibility in burned patients with and without chronic pain and paresthesia problems.* Pain, 1998. **77**(3): p. 241-51.

141. Ward, R.S., et al., *Substance P axons and sensory threshold increase in burn-graft human skin.* J Surg Res, 2004. **118**(2): p. 154-60.

142. Mihara, M., *Regenerated cutaneous nerves in human epidermal and subepidermal regions. An electron microscopy study.* Archives of Dermatological Research, 1984. **276**: p. 115-122.

143. Dertwinkel, R. and et al., *Opioid sensitivity of chronic noncancer pain*, in *Progress in Research and Management*, E. Kalso, et al., Editor. 1999, IASP Press: Seattle. p. 129-141.

144. Patterson, D.R., et al., *The 2002 Lindberg Award. PRN vs regularly scheduled opioid analgesics in pediatric burn patients.* J Burn Care Rehabil, 2002. **23**(6): p. 424-30.

145. Sullivan, S.R., et al., *"Opioid creep" is real and may be the cause of "fluid creep".* Burns, 2004. **30**(6): p. 583-90.

146. Ohrbach, R., et al., *Hypnosis after an adverse response to opioids in an ICU burn patient.* Clin J Pain, 1998. **14**(2): p. 167-75.

147. Sharar, S.R., et al., *A comparison of oral transmucosal fentanyl citrate and oral hydromorphone for inpatient pediatric burn wound care analgesia.* J Burn Care Rehabil, 1998. **19**(6): p. 516-21.

148. Herndon, C.M., et al., *Anticipating and treating opioid-associated adverse effects.* Expert Opin Drug Saf, 2003. **2**(3): p. 305-19.

149. Choinere, M., R. Grenier, and C. Paquette, *Patient-controlled analgesia: A double-blind study in burn patients.* Anaesthesia, 1992. **47**(6): p. 467-472.

150. Finn, J., et al., *A randomised crossover trial of patient controlled intranasal fentanyl and oral morphine for procedural wound care in adult patients with burns.* Burns, 2004. **30**(3): p. 262-8.

151. Sim, K.M., et al., *Use of patient-controlled analgesia with alfentanil for burns dressing procedures: a preliminary report of five patients.* Burns, 1996. **22**(3): p. 238-41.

152. Prakash, S., T. Fatima, and M. Pawar, *Patient-controlled analgesia with fentanyl for burn dressing changes.* Anesth Analg, 2004. **99**(2): p. 552-5.

153. Pasero, C., R.K. Portenoy, and M. McCaffrey, *Opioid analgesics*, in *Pain: Clinical Manual*, M.M.C. Pasero, Editor. 1999, Mosby: St. Louis. p. 161-299.

154. Irving, G.A. and A.D. Butt, *Anaesthesia for burns in children: a review of procedures practised at Red Cross War Memorial Children's Hospital, Cape Town.* Burns, 1994. **20**(3): p. 241-3.

155. Humphries, Y., M. Melson, and D. Gore, *Superiority of oral ketamine as an analgesic and sedative for wound care procedures in the pediatric patient with burns.* J Burn Care Rehabil, 1997. **18**(1 Pt 1): p. 34-6.

156. Mikkelsen, S., et al., *Effect of oral ketamine on secondary hyperalgesia, thermal and mechanical pain thresholds, and sedation in humans.* Reg Anesth Pain Med, 2000. **25**(5): p. 452-8.

157. Schulte, H., A. Sollevi, and M. Segerdahl, *The synergistic effect of combined treatment with systemic ketamine and morphine on experimentally induced windup-like pain in humans.* Anesth Analg, 2004. **98**(6): p. 1574-80.

158. Dadure, C., C. Acosta, and X. Capdevila, *Perioperative pain management of a complex orthopedic surgical procedure with double continuous nerve blocks in a burned child.* Anesth Analg, 2004. **98**(6): p. 1653-5.

159. Cuignet, O., et al., *The efficacy of continuous fascia iliaca compartment block for pain management in burn patients undergoing skin grafting procedures.* Anesth Analg, 2004. **98**(4): p. 1077-81.

160. Pedersen, J.L., et al., *Effect of preemptive nerve block on inflammation and hyperalgesia after human thermal injury.* Anesthesiology, 1996. **84**(5): p. 1020-6.

161. Pedersen, J.L., et al., *Analgesic and anti-inflammatory effects of lignocaine-prilocaine (EMLA) cream in human burn injury.* Br J Anaesth, 1996. **76**(6): p. 806-10.

162. Coleman, L.S., *Bupivacaine and ventricular fibrillation.* Anesthesia and Analgesia, 2004. **99**(4): p. 1269; author reply 1269.

163. Weinberg, G.L., P.H. Hertz, and J. Newman, *Local anesthetic cardiac toxicity can present as late-onset hypotension, bradycardia, and asystole.* Anesthesiology, 2004. **101**(2): p. 553.

164. Jellish, W.S., et al., *Effect of topical local anesthetic application to skin harvest sites for pain management in burn patients undergoing skin-grafting procedures.* Ann Surg, 1999. **229**(1): p. 115-20.

165. Pedersen, J.L., T.S. Galle, and H. Kehlet, *Peripheral analgesic effects of ketamine in acute inflammatory pain.* Anesthesiology, 1998. **89**(1): p. 58-66.

166. Møiniche, S., J.L. Pedersen, and H. Kehlet, *Topical ketorolac has no antinociceptive or anti-inflammatory effect in thermal injury.* Burns, 1994. **20**(6): p. 483-6.

167. Long, T.D., et al., *Morphine-Infused silver sulfadiazine (MISS) cream for burn analgesia: a pilot study.* J Burn Care Rehabil, 2001. **22**(2): p. 118-23.

168. Møiniche, S., J.B. Dahl, and H. Kehlet, *Peripheral antinociceptive effects of morphine after burn injury.* Acta Anaesthesiol Scand, 1993. **37**: p. 710-712.

169. Weinberg, K., et al., *Pain and anxiety with burn dressing changes: patient self-report.* J Burn Care Rehabil, 2000. **21**(2): p. 155-6; discussion 157-61.

170. Patterson, D.R., et al., *Lorazepam as an adjunct to opioid analgesics in the treatment of burn pain.* Pain, 1997. **72**(3): p. 367-74.

171. Everett, J.J., et al., *Adjunctive interventions for burn pain control: comparison of hypnosis and ativan: the 1993 Clinical Research Award.* J Burn Care Rehabil, 1993. **14**(6): p. 676-83.

172. Hansen, S.L., D.W. Voigt, and C.N. Paul, *A retrospective study on the effectiveness of intranasal midazolam in pediatric burn patients.* J Burn Care Rehabil, 2001. **22**(1): p. 6-8.

173. Byers, J.F., et al., *Burn patients' pain and anxiety experiences.* Journal of Burn Care and Rehabilitation, 2001. **22**(2): p. 144-9.

174. Heinrich, M., et al., *Conscious sedation: Off-label use of rectal S(+)-ketamine and midazolam for wound dressing changes in paediatric heat injuries.* Eur J Pediatr Surg, 2004. **14**(4): p. 235-9.

175. Hoffman, H.G., et al., *Water-friendly virtual reality pain control during wound care.* J Clin Psychol, 2004. **60**(2): p. 189-95.

176. Ferguson, S.L. and K.V. Voll, *Burn pain and anxiety: the use of music relaxation during rehabilitation.* J Burn Care Rehabil, 2004. **25**(1): p. 8-14.

177. Fratianne, R.B., et al., *The effect of music-based imagery and musical alternate engagement on the burn debridement process.* J Burn Care Rehabil, 2001. **22**(1): p. 47-53.

178. Prensner, J.D., et al., *Music therapy for assistance with pain and anxiety management in burn treatment.* J Burn Care Rehabil, 2001. **22**(1): p. 83-8; discussion 82-3.

179. Wright, B.R. and P.D. Drummond, *Rapid induction analgesia for the alleviation of procedural pain during burn care.* Burns, 2000. **26**(3): p. 275-82.

180. Martin-Herz, S.P., C.A. Thurber, and D.R. Patterson, *Psychological principles of burn wound pain in children. II: Treatment applications.* J Burn Care Rehabil, 2000. **21**(5): p. 458-72; discussion 457.

181. Frenay, M.C., et al., *Psychological approaches during dressing changes of burned patients: a prospective randomised study comparing hypnosis against stress reducing strategy.* Burns, 2001. **27**(8): p. 793-9.

182. Hoffman, H.G., *Virtual-reality therapy.* Sci Am, 2004. **291**(2): p. 58-65.

183. Hoffman, H.G., et al., *Virtual reality as an adjunctive pain control during burn wound care in adolescent patients.* Pain, 2000. **85**(1-2): p. 305-9.

184. Gaukroger, P.B., *Paediatric analgesia. Which drug? Which dose?* Drugs, 1991. **41**(1): p. 52-9.

185. Gall, O., et al., *Adverse events of premixed nitrous oxide and oxygen for procedural sedation in children.* Lancet, 2001. **358**(9292): p. 1514-5.

186. Stach, D.J., *Nitrous oxide sedation: understanding the benefits and risks.* Am J Dent, 1995. **8**(1): p. 47-50.

187. Day, C.J., J.P. Nolan, and D. Tarver, *Traumatic pneumomyelogram. Implications for the anaesthetist.* Anaesthesia, 1994. **49**(12): p. 1061-3.

188. Old, S., *Management of trauma. Nitrous oxide dangerous in pneumothorax.* Bmj, 1993. **306**(6891): p. 1539.

189. Sharar, S.R., et al., *A comparison of oral transmucosal fentanyl citrate and oral oxycodone for pediatric outpatient wound care.* J Burn Care Rehabil, 2002. **23**(1): p. 27-31.

190. Coyle, N., N. Cherny, and R.K. Portenoy, *Pharmacologic management of cancer pain*, in *Cancer Pain Management*, D. McGuire, Yarbro, C. H., Ferrell, B. R., Editor. 1995, Jones & Bartlett: Boston. p. 89-130.

191. Werner, M.U., et al., *Effects of gabapentin in acute inflammatory pain in humans.* Reg Anesth Pain Med, 2001. **26**(4): p. 322-8.

192. Holthusen, H., S. Irsfeld, and P. Lipfert, *Effect of pre- or post-traumatically applied i.v. lidocaine on primary and secondary hyperalgesia after experimental heat trauma in humans.* Pain, 2000. **88**(3): p. 295-302.

193. Edwards, A.D., *The role of systemic lidocaine in neuropathic pain management.* Journal of Intravenous Nursing, 1999. **22**(5): p. 273-9.

194. Field, T., et al., *Burn injuries benefit from massage therapy.* Journal of Burn Care and Rehabilitation, 1998. **19**(3): p. 241-4.

195. Federal Drug Administration. *Advisory Posted 12/23/04.* 2004 [cited 2005 March 10, 2005]; Available from: http://www.fda.gov/cder/drug/analgesics/default.htm.

Table 1. Mechanisms of Burn Injury

Noxious Stimulus		Mechanism of Action	Example
Thermal	**Conduction**	Most Common. Occurs when a source of heat comes in direct contact with the body	A scald resulting from boiling water
	Radiation	Process by which kinetic energy is transformed into electromagnetic energy until it reaches an object whereupon it converts again to kinetic energy	Ultraviolet rays causing sunburn
	Convection	Refers to air currents that carry heat	Flash injuries that result from a flammable explosion
Chemical		Usually not caused by extreme heat, but rather by tissue reactions to noxious substances	Alkalis, acids, hydrocarbons, and phenols
Electrical		Unique form of conduction burn	Resulting from heat produced by electrical current

Adapted from Rutan, R. L. (1998). Physiologic response to cutaneous burn injury. In G. J. Carrougher (Ed.), *Burn Care and Therapy*, pp. 5-9. St. Louis: Mosby.

Table 2. Classification of Burn Severity

Minor Burn	15% (total body surface area) TBSA or less in adults 10% TBSA or less in children and the elderly 2% TBSA or less full-thickness burn in children or adults without cosmetic or functional risk to eyes, ears, face, hands, feet, or perineum
Moderate Burn	15% to 25% TBSA in adults with less than 10% full-thickness burn 10% to 29% TBSA partial-thickness burn in children under 10 and adults over 40 years of age with less than 10% full-thickness burn 10% TBSA or less full-thickness burn in children or adults without cosmetic or functional risk to eyes, ears, face, hands, feet, or perineum
Major Burn	25% TBSA or greater partial-thickness (dermal) &/or full-thickness burn 20% TBSA or greater in children under 10 and adults over 40 years of age 10% TBSA or greater full-thickness burn All burns involving eyes, ears, face, hands, feet or perineum that are likely to result in cosmetic impairment All high-voltage electrical burns All burn injury complicated by major trauma or inhalation injury All poor risk patients with burn injury

Adapted from Hartford (2002). Care of outpatient burns. In D. N. Herndon (Ed.), *Total Burn Care* (2nd ed.), P. 49. New York: Saunders.

II. Animal Model of Thermal Burn-Induced Primary Mechanical Hyperalgesia

Introduction

The study of inflammation following burn injury has been the focus of a large number of studies, but this literature has largely ignored the problem of pain. There are several reasons that comparatively few studies focus on the mechanisms of burn pain. First, clinical burn pain research is challenging due to the extreme variability in human pain responses, as well as the types and sizes of thermal injuries. Second, human models are limited because the complex cascades of *in vivo* events that contribute to burn pain are difficult to separate in humans. Third, animal models have limited utility to study the mechanisms of thermal burn-induced hyperalgesia.

Animal models of thermal burn injury were developed initially in pigs due to similarities between pig and human skin [1-3], but these models are expensive and do not offer the ease of handling and statistical power of rodent models. The most common rodent models of thermal burn injury involve a full-thickness burn injury [4,5]. A full-thickness burn injury by definition destroys all of the layers of the skin, including structures that are essential for the transduction of pain. In addition, these models involve large thermal injuries of $\geq$ 20% of the animal's total body surface area (TBSA). A full-thickness injury of 15% TBSA has been shown to induce systemic alterations, including profound shifts in fluid, metabolic, and systemic stress responses [6,7]. Such alterations increase the risk of introducing variables that may obfuscate the mechanisms of thermal burn-induced hyperalgesia. Moreover, it is well established that burn pain has specific characteristics among trauma-induced pain due to its intensity and variability both within and between individuals over time [8-17]. Thus, to study burn pain *per se*, a rodent animal model is needed that reliably produces hyperalgesia of both the *magnitude* and the

duration that is consistent with clinical observations. To our knowledge, no such animal model exists.

Recently, a partial-thickness thermal injury of <1% TBSA to the plantar surface of the rat's hind paw was introduced [18]. Two parameters of nociception, thermal and mechanical hyperalgesia, were described in nine studies [18-25] and one textbook chapter [26] that utilized this model.

Jun and Yaksh were the first to describe this model in a study of the facilitation of spinal nociceptive processing generated by persistent small afferent input following tissue injury [27]. The purpose of this study was to characterize the effect of IT gabapentin and two of its structural analogs, S(+)-3-isobutyl γ–aminobutyric acid (GABA) and R(-)-3-isobutyl GABA, on thermal hyperalgesia in a tissue injury model of nociception.

Acute thermal injury was induced by exposing the heel portion of the plantar surface of one hind paw from each rat (n=6) to a metal surface that was heated to 52.5°C for 45 seconds (sec). Thermal hyperalgesia was assessed as paw withdrawal latency (PWL) using a device modeled after that described by Hargreaves [28]. The time interval between the application of the heated metal surface and the hind paw withdrawal response was defined as the PWL. In the absence of a withdrawal response, the stimulus was automatically terminated at 20 sec to avoid tissue injury, and that time was assigned as the maximum response latency (i.e., cut-off time).

To administer the pharmacologic agents, spinal catheters were inserted under halothane anesthesia in male Holtzman Sprague-Dawley rats (300-400 g) 5 to 7 days prior to the thermal injury. Thirty minutes (min) after thermal injury, the response latency in saline-treated rats decreased from 10.5 ± 0.1 sec to 6.35 ± 0.33 sec. The response

latency in the uninjured hind paw was not altered (i.e., PWL 10.9 ± 0.4 sec before injury and 12.9 ± 0.6 sec 30 min after injury). Exposure of the hind paw to a 52.5°C surface for 45 sec resulted in mild erythema and well defined thermal hyperalgesia that returned to baseline after 120 to 150 min. Intrathecal (IT) gabapentin and S(+)-3-isobutyl GABA had no effect on the thermal escape latency of the uninjured hind paw, but produced a well-defined, dose-dependent reversal of the thermal hyperalgesia found in the injured paw, whereas the largest dose (300 μg) of its stereoisomer R(-)-3-isobutyl GABA did not.

In this study, Jun et al (1998) [27] focused on central mechanisms of thermal hyperalgesia following a partial-thickness thermal injury. The PWL test requires that the animal have free movement to exercise a reflexive withdrawal of their hind paw in response to the noxious thermal stimulus. The animal's motor responses may have been influenced for several reasons. First, motor function could have been reduced to some extent by very large doses of gabapentinoids [29,30]. Second, tissue trauma following the insertion of the IT catheters may have stimulated a mechanical hyperalgesia, which could have inhibited the animals' movements. Polyethylene catheters (PE-10) were inserted a full 9 centimeters caudal to the cisterna at the level of the lumbar enlargement. The insertion site for the catheters was through an incision made in the back of the neck and in the atlanto-occipital membrane. While the surgery was accomplished 5 to 7 days prior to thermal injury, mechanical hyperalgesia secondary to tissue injury and/or torsion of the foreign body may have restricted free movement of the animals. Alternatively, movement may have been exaggerated because D-serine has been reported to facilitate the thermally evoked tail flick response when administered alone [31]. However, the authors reported that they did not observe this effect.

It is not clear why PWL returned to baseline after only 3 hours (hrs), because the temperature and duration of exposure to noxious heat were sufficient to cause tissue injury [32]. An inflammatory response took place in the affected tissue as evidenced by erythema. However, it is not possible to determine the actual extent of tissue injury based on macroscopic changes, such as the presence of erythema or the lack of blister and/or edema formation in rat skin [32]. It would be of interest to know if mechanical hyperalgesia, a cardinal sign of inflammation [33], was induced in this model, but these data were not reported.

The second study introduced and validated the model using groups of rats (n=6) that were exposed to temperatures that ranged from 52°C to 52.5°C and duration times that ranged from 15 to 45 sec [18]. Assessments of thermal, as well as mechanical hyperalgesia were performed. A significant decrease in PWL from baseline (i.e., thermal hyperalgesia) was found in the primary area of the injured paw 30 min after injury ($F(6,30)=6.04$, $P<0.0004$). However, no changes in PWLs were found outside the primary area of the injured paw, or in any part of the uninjured hind paw.

Mechanical hyperalgesia was measured using von Frey filaments (Stoelting, Wood Dale, IL) with logarithmically incremental buckling forces of between 0.41 and 15.10 grams (g) that were applied perpendicular to the plantar surface of the hind paw. A differential response was found with 'mild' mechanical hyperalgesia detected in the primary area and 'profound' mechanical hyperalgesia detected in the secondary area on the adjacent uninjured paw. These results suggest that central sensitization contributed to the sensory changes observed in this experiment. However, profound primary mechanical hyperalgesia as well as tactile allodynia have been demonstrated consistently in human

burn models [34-40], which suggests that peripheral sensitization plays a substantial role in both the induction and maintenance of hyperalgesia following thermal injury.

In the third study [23], Jones and Sorkin examined groups of rats (n=5-8) to evaluate the effects of systemic and locally administered gabapentinoids on mechanical hyperalgesia following thermal injury. Similar to Jun et al. [27], they found that gabapentin, which has been used to treat clinical neuropathic pain [41,42], totally blocked secondary mechanical hyperalgesia at a dose of 300 mg/kg, but was associated with severe sedation. In addition, they found that the γ-amino acid analogue, S(+)-3-isobutyl GABA, was even more effective at a lower dose of 100 mg/kg, and had fewer side effects. Von Frey filaments were used to characterize mechanical hyperalgesia in these experiments.

The von Frey filaments were calibrated and administered with the same logarithmically incremental buckling forces as described above. Baseline pre-injury mean mechanical withdrawal thresholds (MWT) were assigned (15 g) for the uninjured heel because the animals did not respond to the maximum von Frey force available (15.1 g), whereas the MWT for the arch was determined to be 14.2 ± 0.4 g. No differences in baseline pre-injury mean MWTs were found between the right and the left hind paws. After thermal injury (52.5°C for 45 sec as previously described), no differences were found between baseline MWT and any other time point for either the injured or the uninjured heel. Thus, primary mechanical hyperalgesia was undetectable following thermal burn in this study.

However, secondary tactile allodynia was observed, as evidenced by a decrease in MWT in the arch area of the injured hind paw (P=0.0001). MWT reached its lowest point

60 min post-burn injury and gradually increased throughout the remainder of the 3 hr experiment. Post hoc testing found that this decrease was significant at all but the 150 min time point following thermal burn. A corresponding significant drop in the mean MWT occurred in the arch of the uninjured hind paw, but it was small in magnitude (i.e., did not go below 10.6 g) compared to baseline (ANOVA, P=0.047). Post-hoc testing revealed there were no significant differences between pre-injury baseline MWT and any other time points.

The authors were unable to explain the inability to detect primary mechanical hyperalgesia in this study. They reasoned that while spinal facilitation presumably explained the presence of secondary tactile allodynia [43], it follows that the intense afferent barrage induced at the time of burn injury would also induce primary mechanical hyperalgesia. Moreover, a low level of continuous input from the injured area is necessary to maintain secondary mechanical hyperalgesia [43]. To be of value in the study of burn pain *per se*, an animal model is needed that produces primary mechanical hyperalgesia similar to that which was found in humans following thermal burn [34-36,38-40].

The fourth study characterized the antihyperalgesic effect of a topical peripheral mu-opioid receptor agonist—loperamide—on thermal burn-induced hyperalgesia in groups of rats (n=5) [19]. Only PWL to a radiant heat source was measured to determine nociceptive threshold. Loperamide was prepared in a cream emulsion of varying doses and applied before and after thermal injury on both hind paws and after injury on the injured paw of morphine tolerant rats. A pharmacokinetic study was also performed.

The thermal burn model yielded a significant primary thermal hyperalgesia, which was attenuated by loperamide in a dose-dependent manner and reversed by naloxone (1mg/kg given intraperitoneally). Rats made tolerant to morphine with subcutaneous morphine pellets were found to exhibit a rightward shift in the dose response curve. The pharmacokinetic study of drug activity in the tissue revealed an elimination half life of 2.3 hrs and a negligible concentration in the blood. Thermal hyperalgesia was found to completely resolve within 3 hrs. Thus, the therapeutic effects of loperamide could not be evaluated because they outlasted thermal hyperalgesia, the only parameter of nociception evaluated in this experiment.

In the fifth study, Sorkin et al. [25] explored the central mechanisms that underlie spinal sensitization and hyperalgesia in groups of rats (n=6-7) following burn injury. Activation of *N*-methyl-D-aspartate (NMDA) receptors by excitatory amino acids is thought to be an essential step in the initiation of central sensitization [44-46]. Indeed, IT administration of NMDA receptor antagonists has been shown to block hyperalgesia [45,47-49]. However, there are two exceptions to this finding. Administration of NMDA receptor antagonists has not blocked hyperalgesia in animal models of acute postoperative pain [50-52] and thermal injury [18,21]. Of note, Meller et al. [53,54] demonstrated that mechanical hyperalgesia was not dependent on NMDA receptor activation, but rather on AMPA receptor activity. Thus, these authors decided to evaluate the effects of Ca^2+ permeable AMPA activity, a non-NMDA-activated receptor, on central sensitization in a model of thermal injury [18,27].

Non-NMDA-activated receptors are divided into classes based on their subunit composition and calcium permeability [55]. Joro spider toxin (JSTX) is a Ca^2+ permeable

AMPA receptor antagonist. Two experiments were conducted. In the first experiment, secondary mechanical hyperalgesia was assessed every 30 min for 3 hrs. At baseline, almost all (17 of 19) of the animals had no response to the stiffest von Frey filament used and were assigned a cutoff of 15 g. Rats that received an injection of 3 μg JSTX 5 min (n=6) or 6 hrs (n=4) prior to thermal injury showed no secondary mechanical hyperalgesia. The efficacy of 3 μg JSTX given 24 hrs (n=8) prior to burn injury was reduced, but withdrawal thresholds were still not significantly different from baseline (i.e., the rats were not allodynic). In the second experiment, secondary thermal hyperalgesia was assessed every 30 min for 2 hrs following thermal injury. No changes were found between baseline and subsequent MWL assessments in response to radiant heat in either the JSTX treated (n=8) or the saline treated group (n=10). These findings suggest that spinal mechanisms associated with secondary mechanical hyperalgesia, but not thermal hyperalgesia, act through calcium permeable AMPA receptors following thermal injury.

The sixth publication features a large series (n=400) of experiments that examined the pharmacology of spinal glutamatergic receptors in post-thermal burn-evoked tactile allodynia and thermal hyperalgesia [20]. Two principle findings were reported from this body of work. First, IT blockade of the NMDA receptor had only a minor or no effect on the subsequent development of primary thermal hyperalgesia and secondary tactile allodynia. This finding was true for competitive and non- competitive NMDA receptor antagonists that were tested. Second, propionic acid-kainate (AMPA-KA) receptor antagonists successfully blocked primary and secondary hyperalgesia and this blockade occurred in a dose-dependent manner at doses that did not cause motor dysfunction.

Combined with their previous studies [18,24,25] and the findings of others [50-52], the authors concluded that distinct spinal excitatory amino acid pharmacology is responsible for the development of secondary tactile allodynia after acute tissue injury and persistent inflammation.

This study is a good example of how this animal model of burn injury can be utilized as a preclinical vehicle for testing therapeutic agents to treat thermal burn-induced hyperalgesia. However, to study the mechanisms of thermal burn-induced hyperalgesia *per se*, the model would need to be modified to mimic the intensity and duration of clinically observed burn pain.

In the next study [20], Nozaki-Taguchi & Yaksh examined the effects of spinal and peripheral mu-opioids on the development of secondary tactile allodynia following thermal injury (n=5). Previously, they had demonstrated that pre-treatment with loperamide (1.7% to 5%) blocked the development of secondary tactile allodynia, whereas post-treatment application did not. In this experiment, pre- and post-treatment with IT morphine (0.1 μg to 10 μg) blocked the development of hyperalgesia following thermal injury. Interestingly, systemically administered naloxone reversed the antiallodynic effect of IT morphine when given before, but not after thermal injury. In addition, a short-acting mu-opioid agonist, remifentanil (3 μg/min), was infused IT peri-injury. Remifentanil did not block subsequent primary thermal hyperalgesia, but did produce a dose-dependent (0.3 μg to 3.0 μg/min) abolition of secondary tactile allodynia. In this experiment, local thermal injury led to a secondary tactile allodynia by the activation of opioid-sensitive afferents and the initiation of a cascade that persisted in the absence of the initial noxious stimulus. However, findings are limited to secondary

mechanical testing for only 3 hrs following thermal injury. Nine rats were excluded from the study due to exclusion criteria set for baseline secondary mechanical thresholds (i.e., <10 g). Yet, the remaining 5 rats were assigned a threshold of 15 g, the maximum von Frey filament stiffness that could be tested. Thus, the range between the minimum and the maximum baseline mechanical thresholds was only 5 g, which resulted in elimination of 60% of the animals. A more sensitive method of testing mechanical nociceptive thresholds may have avoided the elimination of these animals.

In the eighth study [24], Ca^2+-permeable non-NMDA glutamate receptor antagonists were administered in 4 models (n=7 to 9) of inflammatory nociception (i.e., thermal burn, subcutaneous formalin injection, intraplantar administration of carageenan, and allodynia induced by tight ligation of a spinal nerve). Thermal latencies were measured using a Hargreaves box, secondary mechanical nociceptive thresholds were measured using von Frey filaments, and the response to formalin was measured by counting flinches. Both Joro spider toxin (JSTX) and philanthotoxin blocked thermal injury-induced allodynia at spinal doses of 3 μg to 5 μg. The larger dose (5 μg) blocked thermal and mechanical hyperalgesia when given 1 hr after carrageenan but had no effect when given 3 hr after carageenan, in the formalin test or following spinal ligation. However, the lowest dose of JSTX (0.25 μg) at pre-treatment intervals of 60 to 120 min resulted in modest hypoalgesia during thermal testing and at phase 1 of the formalin test. Again, this study focused on central mechanisms. However, most clinical complaints associated with burn pain are due to inflammation [33], which includes peripheral mechanisms (i.e., peripheral sensitization) and von Frey hairs failed to detect primary mechanical hyperalgesia in this model [23].

The final study compared the effects of ketamine and amitriptyline on thermal injury-induced hyperalgesia (n=6-8) [22]. Both pharmacologic agents were injected locally at various doses before and after thermal injury. Hind paw thresholds were determined using a device modeled after Hargreaves and colleagues [28]. Injections of ketamine (100 to 1000 nmol) 15 min before thermal injury produced an antihyperalgesic effect in the ipsilateral paw. When injected prior to thermal injury, amitriptyline attenuated hyperalgesia in a dose dependent manner (300 to 1000 nmol) and induced analgesia at the highest dose. When administered following thermal injury, ketamine had no effect; amitriptyline produced analgesia at the highest dose, but had no effect on hyperalgesia at lower doses (300 to 1000 nmol). Amitriptyline also produced an analgesic effect when injected into the normal contralateral paw (300 and 1000 nmol). Of note, both drugs were found to increase edema in the injured hind paw but by different mechanisms.

These authors found that burn injury induced primary thermal hyperalgesia in the injured paw of the rat. Primary thermal hyperalgesia was most prominent in the first hour, persisted through the second hour, and recovered to baseline by the third hour, as previously observed [19,27].

While the model described by Nozaki-Taguchi et al. [18] has the potential to help elucidate the mechanisms underlying thermal burn-induced hyperalgesia, primary mechanical hyperalgesia has received little attention compared to thermal and secondary mechanical hyperalgesia in the studies conducted to date. Only 2 of the 9 studies reviewed tested for primary mechanical hyperalgesia. Of those, 1 was unable to detect

primary mechanical hyperalgesia [56] and 1 reported it was 'mild' and resolved within 120 to 180 mins following thermal injury [18,22].

However, clinical burn pain evokes primary mechanical hyperalgesia that is characteristically severe, yet variable in intensity, and frequently exacerbated due to repetitious therapeutic procedures over time (i.e., wound debridement, dressing changes, stretching, and range of motion exercises) that are necessary to accomplish healing (i.e., complete reepithelialization of the wound, with minimal loss of range of motion due to deconditioning and scarring). Moreover, a common clinical observation following burn injury is that primary mechanical hyperalgesia often intensifies before healing occurs, a period that can take weeks, months, and sometimes even years [57-60].

Since the studies reviewed above also described an inflammatory response (i.e., erythema and edema), that was noted to last at least 48 hours in some animals, we hypothesized that the limited degree and duration of primary mechanical hyperalgesia might be due to a lack of sensitivity of the experimental design rather than to the thermal burn. If stable long-term mechanical hyperalgesia could be demonstrated, the model would be useful to study the mechanisms that underlie severe and long lasting burn pain. Therefore, the aims of this study were: 1) to modify Nazuki-Taguchi & Yaksh's (1998) model using a more sensitive method to detect primary mechanical hyperalgesia and a more stable temperature stimulus, and 2) to demonstrate changes in thermal burn-induced primary mechanical hyperalgesia over time.

Materials and Methods

Experiments followed American Association of Laboratory Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Francisco (UCSF).

Animals

Twelve male Sprague Dawley rats (Charles River, Hollister, CA), weighing 250 to 300 g at the time that the thermal injury was induced, were housed in pairs in the animal care facility at the University of California, San Francisco under a 12-hour light/dark cycle and were allowed access to standard rat chow and water *ad libitum*. Animal weights, as well as room temperature and humidity, were recorded. All animals were tested during the 12-hour light cycle (7 am to 7 pm) on 12 successive days and then euthanized.

Measurement of Nociceptive Thresholds

Nociceptive threshold was defined as the absolute value of grams of pressure applied before hind paw withdrawal was noted. Nociceptive thresholds were assessed using the model 1601CE(B) anesthesiometer (IITC Life Science, Woodland Hills). The anesthesiometer is similar to the von Frey method of measuring mechanical threshold, but it is a hand-held force transducer adapted with a 0.5 mm^2 polypropylene tip. Similar electronically adapted instruments have been introduced to measure mechanical threshold and have been called an 'electronic pressure algometer' [61] and an 'electronic pressure meter' [62]. We refer to the device used in the current study as an 'electronic von Frey'(EvF).

For the measurement of nociceptive thresholds, rats were placed individually in opaque acrylic cubicles (12.5 x 19 x 15 cm), arranged 6 in a row, back to back, which allowed for the testing of 12 animals. The acrylic cubicles were placed on a table with a wire grid floor 12 cm above a bench surface which provided free access to the hind paws for testing. Mirrors were placed on the bench to facilitate visualization of the plantar surface of the hind paws. To decrease variability, nociceptive threshold training was conducted to familiarize the animals with the experimental setting and testing procedures [63] for 4 successive days prior to thermal injury.

On the first day of training, animals were brought to a quiet room specifically designated for animal behavioral testing and allowed to adapt to the environment for a couple of hours undisturbed in their housing cages.

On the second day of training, animals were brought to the same room, gently removed from their housing cages and placed individually into the acrylic testing cubicles. Animals were allowed to adapt for 1 hour undisturbed. During this adaptation period, the animals were observed to engage in spontaneous exploration and grooming activity, after which the animals appeared calm. Following the adaptation period, the animals underwent testing with the EvF. Testing consisted of placing the tip of the EvF perpendicular to the heel of each hind paw, beginning with the left hind paw. During this initial exposure to the EvF, hind paws were tested twice at 6 minute intervals.

On the third day of training, the procedure was repeated, but animals underwent 4 EvF assessments, alternating between the left and the right hind paw every 6 min. The animals remained in the acrylic cubicles for another hour prior to being returned to the animal care facility.

On the fourth day, training was identical except that, after nociceptive threshold testing with the EvF was completed, animals were retested in 1 hour and then returned to the animal care facility.

Thermal Burn

After baseline nociceptive thresholds were assessed on both hind paws, the rat was placed in an induction box with isofluorane 2.5 to 5% delivered in a 50:50 mixture of oxygen and room air. With the loss of spontaneous movement, lack of response to a firm pinch on the forepaw, and lack of a corneal reflex, the animal was gently removed from the induction chamber and anesthesia was maintained with isoflurane delivered by nose cone. Thermal injury was induced by placing the plantar surface of the heel of the right hind paw on a brass thermal probe with a surface temperature of 52.5±0.1°C. A 9-gram sand pouch was placed on the dorsum of the hind paw to maintain constant, gentle pressure to the heel area on the brass probe during thermal exposure. Paw exposure was maintained for 45 seconds. After this, the animal was placed supine on a bed of wood shavings in its housing cage to recover. Spontaneous activity was typically observed within 3 min of removal from anesthesia. Animals were then placed in the acrylic testing cubicles to adapt prior to nociceptive threshold testing with the EvF. Nociceptive threshold measurements were obtained as described above at 0.5, 1, 2, 3, 4, 6, and 8 hrs, and 1, 2, 3, 4, 5, 6, and 7 days (i.e., 24, 48, 72, 96, 120, 144, and 168 hrs) following burn injury.

Statistical Analyses

Nociceptive thresholds were determined by averaging 4 measurements with the EVF for each time point. Time course data are presented as mean nociceptive thresholds in grams (± SEM).

A paired t-test was used to evaluate for differences in baseline nociceptive thresholds between the 2 paws. A 2-way, repeated measures analysis of variance (ANOVA) with 2 within subjects' factors (i.e., treatment and time) was used to determine if there were differences over time in nociceptive thresholds between the uninjured and injured paws. A P-value of <0.05 was considered statistically significant.

Post hoc contrasts were used to examine for differences in nociceptive thresholds at each time point compared to baseline. Additional within group contrasts were done to determine differences in nociceptive thresholds between time points. Finally, between group contrasts were done at each time point. For all of the contrasts the alpha level was set at $P < 0.003$ (i.e., 0.05 overall alpha ÷ 15 nociceptive threshold assessment time points). These data were analyzed using SPSS®.

Results

Erythema and edema were noted in the injured paw within 5 min in a few animals following burn injury, but there was no change in skin integrity and no blister formation. Local erythema and edema were transient, lasting up to 48 hours. All animals appeared to bear weight normally on both the injured and uninjured paws. They also exhibited exploratory and grooming behavior, gained weight, and never guarded the injured paw, except occasionally when the paw was touched during testing.

Comparison of Baseline Nociceptive Thresholds

No differences were found in pre-injury baseline nociceptive thresholds between injured paws (95.35 ± 2.93 g) and the uninjured paws (94.59 ± 3.05 g; t=0.75, P=0.47).

Overall Differences in Nociceptive Thresholds Over Time

The 2-way, repeated measures ANOVA demonstrated a significant treatment x time interaction ($F(14,154)=14.75$, $P=<0.0001$), as well as a main effect of paw treatment group ($F(1,11)=484.02$, $P<0.0001$) and time ($F(14,154)=37.23$, $P=<0.0001$). As shown in Figure 1, these results indicate that, overall, the injured paw demonstrated significantly lower thresholds and that the differences in nociceptive thresholds between the injured and the uninjured paws were dependent on time. To further explore these differences, a series of post hoc contrasts were performed.

Within Group Changes in Nociceptive Thresholds Compared to Baseline

Injured paw

As shown in Table 1, tests of within subjects' effects that compared nociceptive thresholds in the injured paw, at each time point, to baseline thresholds revealed significantly lower nociceptive thresholds at all of the time points tested (all $P<0.0001$).

Uninjured paw

As shown in Table 1, tests of within subjects effects that compared nociceptive thresholds in the uninjured paw, at each time point, to baseline thresholds revealed significantly lower nociceptive thresholds at 6 hrs ($P=0.001$), 8 hrs ($P<0.001$), 2 days ($P<0.0001$) and 3 days ($P<0.0001$) following thermal injury.

Within Group Changes in Nociceptive Thresholds Over Time

Injured paw

A repeated measures ANOVA demonstrated a significant main effect of time ($F(14,154)=62.80$, $P<0.0001$). When each time point was compared to the previous time point, significant differences were found for baseline versus 0.5 hr ($P<0.0001$), 4 hrs versus 6 hrs ($P=0.002$), and 6 days versus 7 days ($P<0.0001$).

Uninjured paw

A repeated measures ANOVA demonstrated a significant main effect of time ($F(14,154)=4.83$, $P<0.0001$). When each time point was compared to the previous time point, no significant differences in nociceptive thresholds were found for any of the time points.

Between Group Differences at Each Time Point

Post hoc contrasts that compared nociceptive thresholds between the injured and uninjured paws at each time point found significant differences at every time point (0.5, 1, 2, 3, 4, 6, and 8 hrs, and 1, 2, 3, 4, 5, 6, and 7 days following thermal injury; all $P<0.0001$) except baseline (Fig 1). These findings indicated that nociceptive thresholds in the injured paw were significantly lower than the uninjured paw at every time point that was measured except baseline.

Discussion

This study is the first to demonstrate long-lasting primary mechanical hyperalgesia following a partial-thickness burn injury. Findings from this study contrast with studies that were unable to detect [25] or reported 'mild' transient primary

mechanical hyperalgesia following the same thermal stimulus [18,23]. One explanation for these differences is that previous studies used nylon monofilaments to measure nociceptive thresholds. The EvF may be a more sensitive method for detecting mechanical hyperalgesia than nylon monofilaments because the gradient of pressure is continuous. This approach contrasts with nylon monofilaments that increase in intensity in 0.22 log increments [64].

A marked 60% decrease in nociceptive thresholds (Fig. 1) was found at the first testing time point (i.e., 0.5 hours following injury). This profound level of hyperalgesia persisted for 4 hours and then intensified further 6 hours post-thermal injury and persisted for at least 6 more days. Nociceptive thresholds increased 16% between day 6 and day 7, but did not return to baseline thresholds.

An animal model of postoperative inflammatory pain also demonstrated an increase in primary mechanical hyperalgesia 1 to 3 days following surgical incision of the plantar surface of the rat hind paw [65]. However, these animals were not tested for longer than 3 days; and no further comparisons can be made between the models. The local release of inflammatory mediators and the accumulation of immune cells that are attracted to the wound to protect against infection and promote wound healing likely contribute to the magnitude, as well as the duration, of primary mechanical hyperalgesia following burn injury [33]. However, the exact mechanisms that contribute to primary mechanical hyperalgesia following burn injury remain to be elucidated. In addition, the time course to recovery in burn-injured tissue requires investigation in correlation with changes in primary mechanical hyperalgesia.

While the EvF is more sensitive at detecting nociceptive thresholds than traditional von Frey filaments, it does have some limitations. Like traditional von Frey filaments, the EvF is a hand-held instrument. Thus, the angle, ramp, and force cannot be uniformly controlled by human manipulation, increasing the potential for variability. However, by averaging 4 nociceptive thresholds per measurement time point, variability was decreased.

A small decrease in nociceptive threshold was also observed in the uninjured paw, which may be due to some degree of mechanical hyperalgesia. Contralateral spread of neural excitation resulting in mirror image pain has been known to develop following tissue injury in animals [66-69] and humans [70,71]. Moreover, remote hyperalgesia has been reported following thoracic burn injury in rats [72,73]. Thus, the decrease in nociceptive threshold in the uninjured paw may be due to mirror image hyperalgesia. However, the decrease in nociceptive threshold in the uninjured paw was minimal (range -2% to -22% from baseline) and never approached the magnitude observed in the injured paw (range -54% to -76% from baseline).

Conclusion

As modified, this model is the first to provide a robust, practical, well-integrated biological system to reliably measure the time course of thermal burn-induced primary mechanical hyperalgesia. This approach is important because primary mechanical hyperalgesia, evoked by painful therapeutic procedures, is the most severe and difficult type of clinical burn pain to manage. Modifying the model by using with the EvF makes it possible for the first time to study the mechanisms that underlie both the magnitude and

the duration of primary mechanical hyperalgesia over the natural course of burn recovery. Thus, the model represents a unique and valuable contribution to the study of burn pain. Elucidating the mechanisms that contribute to burn pain is critically important to the development of analgesic therapies that are so desperately needed by this vulnerable population.

Acknowledgements

Thank you to Dr. Linda Sorkin, Professor of Anesthesiology, University of California, San Diego (CA) for generously providing information and materials that was helpful in assembling the thermal stimulus device for this model. This work was supported by a predoctoral Ruth L. Kirschstein National Research Service Award #5 F31 NR07474, from the National Institute for Nursing Research, National Institutes of Health, Bethesda, MD (GJS).

References

1. Behounkova-Houskova, E., Moserova, J., *Histology of healing of deep dermal burns in pigs.* Scandinavian Journal of Plastic Reconstructive Surgery, 1979. **13**(1): p. 57-60.
2. Cruz, N.I., Bayron, F. E., Suarez, A. J., *Accelerated healing of full-thickness burns by the use of high-voltage pulsed galvanic stimulation in the pig.* Annals of Plastic Surgery, 1989. **23**(1): p. 49-55.
3. Brink, J.A., Sheets, P. W., Dines, K. A., Etchison, M. R., Hanke, C. W., Sadove, A. M., *Quantitative assessment of burn injury in porcine skin with high-frequency ultrasonic imaging.* Investigative Radiology, 1986. **21**(8): p. 645-651.
4. Schildt, B., Nilsson, A., *Standardized burns in mice.* European Surgical Research, 1970. **2**: p. 23-33.
5. Walker, H.L., Mason Jr., A. D., *A standardized animal burn.* The Journal of Trauma, 1968. **8**(6): p. 1049-1051.
6. Demling, R.H., *Burns.* New England Journal of Medicine, 1985. **313**: p. 1389-1398.
7. Osgood, P.F., Murphy, J. L., Carr, D. B., Szyfelbein, S. K., *Increases in plasma beta-endorphin and tail flick latency in the rat following burn injury.* Life Sciences, 1987. **40**: p. 547-554.
8. Chapman, C.R., Turner, J.A., *Psychological control of acute pain in medical settings.* Journal of Pain Symptom Management, 1986. **1**: p. 9-20.

9. Choinière, M., Melzack, R., Girard, N., Rondeau, J., Paquin, M. J., *Comparisons between patients' and nurses' ratings of pain and medication efficacy in severe burn injuries.* Pain, 1990. **40**: p. 143-152.

10. Difede, J., et al., *Determinants of pain expression in hospitalized burn patients.* Pain, 1997. **72**(1-2): p. 245-51.

11. Geisser, M.E., Bingham, H. G., Robinson, M. E., *Pain and anxiety during burn dressing changes: concordance between patients' and nurses' ratings and relation to medication administration and patient variables.* Journal of Burn Care and Rehabilitation, 1995. **16**: p. 165-171.

12. Gilboa, D., Friedman, M, & Tsur, H, *The burn as a continuous traumatic stress: Implications for emotional treatment during hospitalization.* Journal of Burn Care and Rehabilitation, 1994. **15**: p. 86-91.

13. Malenfant, A., et al., *Tactile, thermal and pain sensibility in burned patients with and without chronic pain and paresthesia problems.* Pain, 1998. **77**(3): p. 241-51.

14. Patterson, D.R., et al., *Lorazepam as an adjunct to opioid analgesics in the treatment of burn pain.* Pain, 1997. **72**(3): p. 367-74.

15. Perry, S., *Undermedication for pain on a burn unit.* General Hospital Psychiatry, 1984. **6**: p. 308-316.

16. Taal, L.A. and A.W. Faber, *Burn injuries, pain and distress: exploring the role of stress symptomatology.* Burns, 1997. **23**(4): p. 288-90.

17. Ulmer, J.F., *An exploratory study of pain, coping, and depressed mood following burn injury.* J Pain Symptom Manage, 1997. **13**(3): p. 148-57.

18. Nozaki-Taguchi, N. and T.L. Yaksh, *A novel model of primary and secondary hyperalgesia after mild thermal injury in the rat.* Neurosci Lett, 1998. **254**(1): p. 25-8.

19. Nozaki-Taguchi, N. and T.L. Yaksh, *Characterization of the antihyperalgesic action of a novel peripheral mu-opioid receptor agonist--loperamide.* Anesthesiology, 1999. **90**(1): p. 225-34.

20. Nozaki-Taguchi, N. and T.L. Yaksh, *Spinal and peripheral mu opioids and the development of secondary tactile allodynia after thermal injury.* Anesth Analg, 2002. **94**(4): p. 968-74, table of contents.

21. Nozaki-Taguchi, N. and T.L. Yaksh, *Pharmacology of spinal glutamatergic receptors in post-thermal injury-evoked tactile allodynia and thermal hyperalgesia.* Anesthesiology, 2002. **96**(3): p. 617-26.

22. Oatway, M., A. Reid, and J. Sawynok, *Peripheral antihyperalgesic and analgesic actions of ketamine and amitriptyline in a model of mild thermal injury in the rat.* Anesth Analg, 2003. **97**(1): p. 168-73, table of contents.

23. Jones, D.L. and L.S. Sorkin, *Systemic gabapentin and S(+)-3-isobutyl-gamma-aminobutyric acid block secondary hyperalgesia.* Brain Res, 1998. **810**(1-2): p. 93-9.

24. Sorkin, L.S., T.L. Yaksh, and C.M. Doom, *Pain models display differential sensitivity to Ca2+-permeable non-NMDA glutamate receptor antagonists.* Anesthesiology, 2001. **95**(4): p. 965-73.

25. Sorkin, L.S., T.L. Yaksh, and C.M. Doom, *Mechanical allodynia in rats is blocked by a Ca2+ permeable AMPA receptor antagonist.* Neuroreport, 1999. **10**(17): p. 3523-6.

26. Allen, J.W. and T.L. Yaksh, *Assessment of acute thermal nociception in laboratory animals*, in *Pain Research: Methods and Protocols*, Z.D. Luo, Editor. 2004, Humana Press Inc.: Totowa NJ. p. 11-23.

27. Jun, J.H. and T.L. Yaksh, *The effect of intrathecal gabapentin and 3-isobutyl gamma-aminobutyric acid on the hyperalgesia observed after thermal injury in the rat.* Anesth Analg, 1998. **86**(2): p. 348-54.

28. Hargreaves, K., Dubner, R., Brown, F., Flores, C., Joris, J., *A new sensitive method for measuring thermal nociception in cutaneous hyperalgesia.* Pain, 1988. **32**: p. 77-88.

29. Partridge, B.J., et al., *Characterization of the effects of gabapentin and 3-isobutyl-γ-aminobutyric acid on substance P-induced thermal hyperalgesia.* Anesthesiology, 1998. **88**: p. 196-205.

30. Hunter, J.C., et al., *The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain.* European Journal of Pharmacology, 1997. **324**: p. 153-160.

31. Kolhekar, R., S.T. Meller, and G.F. Gebhart, *N-methyl-D-aspartate receptor-mediated changes in thermal nocicepton.* Neuroscience, 1994. **63**: p. 925-936.

32. Suzuki, T., et al., *Experimental studies of moderate temperature burns.* Burns, 1991. **17**(6): p. 443-451.

33. Levine, J.D., Reichling, D B, *Peripheral mechanisms of inflammatory pain*, in *Textbook of Pain*, P.D. Wall, Melzack, R, Editor. 1999, Churchill Livingstone: New York. p. 59-84.

34. Dahl, J.B., et al., *The effect of pre- versus postinjury infiltration with lidocaine on thermal and mechanical hyperalgesia after heat injury to the skin.* Pain, 1993. **53**(1): p. 43-51.

35. Pedersen, J.L. and H. Kehlet, *Hyperalgesia in a human model of acute inflammatory pain: a methodological study.* Pain, 1998. **74**(2-3): p. 139-51.

36. Raja, S.N., J.N. Campbell, and R.A. Meyer, *Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin.* Brain, 1984. **107 (Pt 4)**: p. 1179-88.

37. Meyer, R.A. and J.N. Campbell, *Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand.* Science, 1981. **213**(4515): p. 1527-9.

38. LaMotte, R.H., et al., *Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat.* The Journal of Neuroscience, 1982. **2**(6): p. 765-781.

39. LaMotte, R.H., et al., *Time-intensity profiles of cutaneous pain in normal and hyperalgesic skin: a comparison with C-fiber nociceptor activities in monkey and human.* J Neurophysiol, 1984. **51**(6): p. 1434-50.

40. Møiniche, S., J.B. Dahl, and H. Kehlet, *Time course of primary and secondary hyperalgesia after heat injury to the skin.* Br J Anaesth, 1993. **71**(2): p. 201-5.

41. Mellick, G.A. and G.B. Mellick, *Reflex sympathetic dystrophy treated with gabapentin.* Archives of Physiology and Medical Rehabilitation, 1997. **78**: p. 98-105.

42. Rosner, H., L. Rubin, and A. Kestenbaum, *Gabapentin adjunctive therapy in neuropathic pain states.* Clinical Journal of Pain, 1996. **12**: p. 56-58.

43. LaMotte, R.H., et al., *Neurogenic hyperalgesia: Psychophysical studies of underlying mechanisms.* Journal of Neurophysiology, 1991. **66**: p. 190-211.

44. Coderre, T.J. and R. Melzack, *The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury.* Journal of Neuroscience, 1992. **12**: p. 3665-3670.

45. Yamamoto, T. and T.L. Yaksh, *Comparison of the antinociceptive effects of pre- and post-treatment with intrathecal morphine or MK801, an NMDA antagonist, on the formalin test in the rat.* Anesthesiology, 1992. **77**: p. 757-763.

46. Haley, J.E., A.F. Sullivan, and A.H. Dickenson, *Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat.* Brain Research, 1990. **518**: p. 218-226.

47. Ren, K. and R. Dubner, Neuroscience Letters, 1993. **163**: p. 22-26.

48. Dougherty, P.M., et al., Journal of Neuroscience, 1992. **12**(3025-3041).

49. Dickenson, A.H. and A.F. Sullivan, Brain Research, 1990. **506**: p. 31-39.

50. Pogatzki, E.M., P.K. Zahn, and T.J. Brennan, *Effect of pretreatment with intrathecal excitatory amino acid receptor antagonists on the development of pain behavior caused by plantar incision.* Anesthesiology, 2000. **93**: p. 489-496.

51. Zahn, P.K. and T.J. Brennan, Anesthesiology, 1998. **88**: p. 143-156.

52. Zahn, P.K., E. Umali, and T.J. Brennan, Pain, 1998. **74**: p. 213-223.

53. Meller, S.T., C.L. Dykstra, and G.F. Gebhart, *Acute mechanical hyperalgesia is produced by coactivation of AMPA and metabotropic glutamate receptors.* Neuroreport, 1993. **4**(7): p. 879-82.

54. Meller, S.T., C. Dykstra, and G.F. Gebhart, *Acute mechanical hyperalgesia in the rat can be produced by coactivation of spinal ionotropic AMPA and metabotropic glutamate receptors, activation of phospholipase A2 and generation of cyclooxygenase products.* Prog Brain Res, 1996. **110**: p. 177-92.

55. Hollman, M., M. Hartely, and S. Heinemann, Science, 1991. **252**: p. 851-853.

56. Jones, T.L. and L.S. Sorkin, *Calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptors mediate development, but not maintenance, of secondary allodynia evoked by first-degree burn in the rat.* J Pharmacol Exp Ther, 2004. **310**(1): p. 223-9.

57. Silbert, B.S., Osgood, P F, Carr, D B, *Burn pain*, in *Anesthesia: Biologic Foundations*, T. Yaksh, Lynch III, C, Zapol, W M, Maze, M, Biebuyck, J F, Saidman, L J, Editor. 1999, Lippincott-Raven: Philadelphia.

58. Latarjet, J. and M. Choinière, *Pain in burn patients.* Burns, 1995. **21**(5): p. 344-8.

59. Choinière, M., Melzack, R, Rondeau, J., et al., *The pain of burns: Characteristics and correlates.* Journal of Trauma, 1989. **29**: p. 1531-1539.

60. Choinière, M., *Burn pain: A unique challenge.* Pain--Clinical Updates, 2001(Spring).

61. Möller, K.Ä., B. Johansson, and O.-G. Berge, *Assessing mechanical allodynia in the rat paw with a new electronic algometer.* Journal of Neuroscience Methods, 1998. **84**: p. 41-47.

62. Parada, C.A., et al., *Tumor necrosis factor receptor type-1 in sensory neurons contributes to induction of chronic enhancement of inflammatory hyperalgesia in rat.* Eur J Neurosci, 2003. **17**(9): p. 1847-52.

63. Taiwo, Y.O., T.J. Coderre, and J.D. Levine, *The contribution of training to sensitivity in the nociceptive paw-withdrawal test.* Brain Research, 1989. **487**(1): p. 148-151.

64. Levin, S., G. Pearsall, and R.J. Ruderman, *Von Frey's method of measuring pressure sensibility in the hand: an engineering analysis of the Weinstein-Semmes pressure aesthesiometer.* J Hand Surg [Am], 1978. **3**(3): p. 211-6.

65. Whiteside, G.T., et al., *Pharmacological characterisation of a rat model of incisional pain.* Br J Pharmacol, 2004. **141**(1): p. 85-91.

66. Coderre, T.J. and R. Melzack, *Cutaneous hyperalgesia: contributions of the peripheral and central nervous systems to the increase in pain sensitivity after injury.* Brain Res, 1987. **404**(1-2): p. 95-106.

67. Milligan, E.D., et al., *Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats.* J Neurosci, 2003. **23**(3): p. 1026-40.

68. Oaklander, A.L. and J.M. Brown, *Unilateral nerve injury produces bilateral loss of distal innervation.* Annals of Neurology, 2004. **55**(5): p. 639-644.

69. Milligan, E.D., S.F. Maier, and L.R. Watkins, *Sciatic inflammatory neuropathy in the rat: surgical procedures, induction of inflammation, and behavioral testing.* Methods Mol Med, 2004. **99**: p. 67-89.

70. Bowsher, D., *Contralateral mirror-image pain following anterolateral cordotomy.* Pain, 1988. **33**(1): p. 63-5.

71. Nagaro, T., et al., *New pain following cordotomy: clinical features, mechanisms, and clinical importance.* J Neurosurg, 2001. **95**(3): p. 425-31.

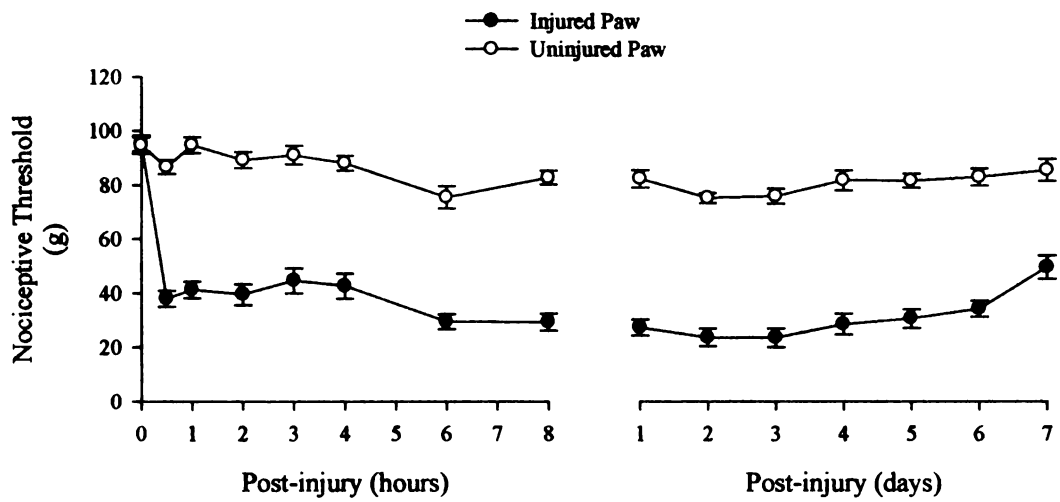
72. Ueda, M., et al., *Foot hyperalgesia after thoracic burn injury--histochemical, behavioral and pharmacological studies.* Acta Histochem Cytochem, 2001. **34**: p. 441-450.

73. Ueda, M., et al., *Nerve growth factor induces systemic hyperalgesia after thoracic burn injury in the rat.* Neurosci Lett, 2002. **328**(2): p. 97-100.

Table 1. Pairwise within-subjects contrasts comparing differences in nociceptive thresholds between baseline and all other nociceptive threshold testing time points following burn injury (mean grams ± SEM). Bolded font denotes statistical significance ($P \leq 0.003$)

Time Following Burn	**Hrs**	**0**	**0.5**	**1**	**2**	**3**	**4**	**6**	**8**							
	Day									**1**	**2**	**3**	**4**	**5**	**6**	**7**
Nociceptive Threshold *Uninjured* paw		95 ±3g	87 ± 3g	95 ± 3g	89 ± 3g	91 ± 3g	88 ± 3g	**75 ± 4g**	**83 ± 3g**	82 ± 3g	**75 ± 2g**	**76 ± 3g**	82 ± 4g	81 ± 3g	83 ± 3g	85 ± 4g
Decrease *Uninjured* paw			10 ±5%	2 ±5%	9 ±4%	6 ±5%	8 ±6%	**22 ±5%**	**15 ±4%**	15 ±5%	**22 ±4%**	**21 ±5%**	16 ±4%	16 ±5%	15 ±5%	11 ±6%
Nociceptive Threshold *Injured* paw		95 ±3g	**38 ±3g**	**41 ±3g**	**39 ±4g**	**45 ±5g**	**43 ±5g**	**29 ±3g**	**2 ±3g**	**27 ±3g**	**24 ±3g**	**23 ±3g**	**28 ±4g**	**30 ±3g**	**34 ±3g**	**50 ±4g**
Decrease *Injured* paw			**60 ±3%**	**57 ±3%**	**59 ±1%**	**54 ±4%**	**56 ±4%**	**69 ±3%**	**70 ±3%**	**71 ±3%**	**76 ±3%**	**76 ±3%**	**70 ±4%**	**68 ±4%**	**64 ±3%**	**48 ±4%**

Fig. 1. Thermal burn-induced hyperalgesia compared between injured and uninjured paw groups as measured by a decrease in nociceptive threshold (mean grams ± SEM). Post-hoc interaction contrasts between time x group nociceptive thresholds were significant at all timepoints compared to baseline (all $P<0.0001$).



Text from Section II has been included in Section III of this dissertation to be submitted for publication in *PAIN*®, the Journal of the International Association for the Study of Pain. If accepted, permission to publish the manuscript from my dissertation will be obtained by Elsevier.

Thermal Burn-Induced Mechanical Hyperalgesia in the Rat Involves TrkA and PKCε Signaling

Gretchen J. Summer, R.N., Ph.D. [a,b,c*]

Kathleen A. Puntillo, R.N., D.N.Sc., F.A.A.N. [a]

Christine Miaskowski, R.N., Ph.D., F.A.A.N. [a]

Paul G. Green, Ph.D. [b,c,d]

Jon D. Levine, M.D., Ph.D. [a,b,c,d]

[a] Department of Physiological Nursing, School of Nursing

[b] NIH Pain Center (UCSF)

[c] Department of Oral and Maxillofacial Surgery, School of Dentistry

[d] Division of Neuroscience, Biomedical Sciences Program

University of California-San Francisco

* Corresponding author. Address: NIH Pain Center (UCSF), Neuroscience and Biomedical Sciences Programs, University of California-San Francisco, 521 Parnassus Avenue, San Francisco, CA 94143, USA. Tel.: +1 415 476 0604; fax +1 415 476 6305. *E-mail address*: Gretchen.Summer@ucsf.edu (G. J. Summer).

Abstract

While mechanical hyperalgesia associated with procedures is the major source of severe pain in burn-injured patients, little is known about its underlying mechanism. One reason for this has been the lack of a model for mechanical hyperalgesia at the site of thermal burn injury, primary mechanical hyperalgesia. To address this, we made two modifications to an established rat thermal burn injury model (Nozaki-Taguchi & Yaksh, 1998): 1) use of a more constant thermal stimulus to stabilize burn severity and 2) use of an electronic von Frey hair stimulator to increase measurement sensitivity. In this model, primary mechanical hyperalgesia is present from the first measurement 0.5 hours following injury, reaches a maximum 2 to 4 days, and is still significant 7 days post-burn. Since nerve growth factor (NGF), which is elevated in burn-injured tissue, produces mechanical hyperalgesia and activates protein kinase C-epsilon (PKCε), a key mediator in inflammatory and neuropathic pain, we used this model to evaluate the role of the high affinity NGF receptor, tyrosine kinase A (TrkA), and PKCε, in thermal burn-induced primary mechanical hyperalgesia. Intrathecal administration of antisense oligodeoxynucleotides (ODN) to these two candidate molecules, prior to inducing a superficial partial-thickness burn, caused a dose-related decrease in primary mechanical hyperalgesia. Thus, our thermal burn model provides a method to elucidate the mechanism as well as to screen for analgesic treatment of this important clinical pain condition.

Introduction

Burn injury induces severe pain that is difficult to manage [1-14]. The magnitude of this problem is suggested by the fact that in the United States alone at least 1.25 million individuals annually seek medical care for a serious burn injury [15]. Moreover, pain may contribute to sensory problems, including cold allodynia and peripheral neuropathy [6,16-18], and psychological disorders (e.g., post-traumatic stress disorder [PTSD]) [5,9,19-23], which commonly develop following burn injury.

Although potent analgesics such as opioids are frequently administered to patients with burn injury pain, even large doses are often ineffective [12]. Moreover, opioids induce adverse effects (i.e., nausea, sedation, immunosuppression, hypotension, decreased appetite and dysphoria) that impede recovery in burn-injured patients [24-28].

While novel agents that target burn injury pain are critically needed, animal models to study this problem often fail to demonstrate nociceptor sensitization to mechanical stimuli at the site of thermal injury *in vitro* [29-33] or touch-evoked pain at the site of thermal injury, primary mechanical hyperalgesia, *in vivo* [34]. This contrasts with well-established human studies that demonstrate primary mechanical hyperalgesia following a noxious heat stimulus applied to the skin [35-42]. Thus, an objective of this study was to develop an animal model of thermal burn-induced primary mechanical hyperalgesia that mimics the intensity and duration of clinically observed burn pain.

A second objective was to use this new model to elucidate mechanisms that underlie thermal burn-induced primary mechanical hyperalgesia. Nerve growth factor (NGF), which is elevated in burn-injured tissue [43-45], produces mechanical hyperalgesia [46-49]. It acts preferentially at the tyrosine kinase A receptor (TrkA), the high affinity receptor for NGF, via four well-described second messenger pathways: extracellular signal-related kinase/mitogen-activated protein kinase (ERK/MEK) [50-52], phosphatidylinositol 3-kinase

(P13K)[53-55], phospholipase Cγ (PLCγ) [56,57] and protein kinase C-epsilon (PKCε) [58,59]. PKCε is a second messenger whose function has a close relationship with the production of inflammatory hyperalgesia and the sensitization of nociceptors [60]. In particular, PKCε is principally responsible for sensitization of the heat response in nociceptors by bradykinin [61]. NGF can activate PKCε *in vitro* [62] and *in vivo* [63]. These observations led us to test the hypothesis that NGF, acting at TrkA receptors, and PKCε contribute to thermal burn-induced primary mechanical hyperalgesia.

Materials and Methods

Experiments followed American Association of Laboratory Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Francisco (UCSF).

Animals

Male Sprague Dawley rats (Charles River, Hollister, CA), weighing 250 to 300 g at the time that the thermal burn was induced, were housed in pairs in the animal care facility at the University of California, San Francisco under a 12-hour light/dark cycle and were allowed access to standard rat chow and water *ad libitum*. Animal weights, as well as room temperature and humidity, were recorded. All animals were tested during the 12-hour light cycle (7 am to 7 pm) on successive days and then euthanized according to UCSF IACUC policy regulations. All efforts were made to minimize the number of animals used and their discomfort.

Measurement of Nociceptive Thresholds

Nociceptive threshold was defined as the applied pressure, in grams, needed to elicit hind paw withdrawal (HPW). Nociceptive thresholds were assessed using the model 1601CE(B) anesthesiometer (IITC Life Science, Woodland Hills, CA). The anesthesiometer is similar to the von Frey method of measuring mechanical threshold, but it is a hand-held force transducer adapted with a 0.5 mm^2 polypropylene tip. We chose to use the electronic method over nylon monofilaments because it provides a continuous gradient of pressure and, thus, is a more sensitive method for detecting mechanical hyperalgesia. This approach contrasts with nylon monofilaments that increase in intensity in 0.22 log increments. Others have used electronically adapted instruments to measure mechanical threshold, referring to the device as an 'electronic pressure algometer' [64] and an 'electronic pressure meter' [65-67]. We refer to the device used in the current study as an electronic von Frey (EvF).

For the measurement of nociceptive thresholds, rats were placed individually in opaque acrylic cubicles (12.5 x 19 x 15 cm), arranged 6 in a row, in two rows, back to back; which allowed for the testing of 12 animals. The acrylic cubicles were placed on a table with a wire grid floor, 12 cm above a bench surface, which provided free access to the hind paws for testing. Mirrors were placed on the bench to facilitate visualization of the plantar surface of the hind paws during testing. To decrease variability, nociceptive threshold training was conducted to familiarize the animals with the experimental setting and testing procedures [68] for 4 successive days prior to thermal injury.

On the first day of training, animals were brought to a quiet room, specifically designated for animal behavioral testing, and allowed to adapt to the environment for a couple of hours undisturbed in their housing cages. On the second day of training, animals were brought to the same room, removed from their housing cages and placed individually into the acrylic testing cubicles. Animals were allowed to adapt for 1 hour undisturbed.

During this adaptation period, the animals were observed to engage in spontaneous exploration and grooming activity, after which the animals appeared calm. Following the adaptation period, the animals underwent training and testing with the EvF. Testing consisted of placing the tip of the EvF perpendicular to the heel of each hind paw, beginning with the left hind paw. During this initial exposure to the EvF, hind paws were tested twice at 6 minute (min) intervals.

On the third day of training, the procedure was repeated, but animals underwent 4 measurements, alternating between the left and the right hind paw every 6 min until HPW was noted. The animals were then allowed to remain in the acrylic cubicles for another hour prior to being returned to their housing cages and then to the animal care facility. On the fourth day, training was identical, except that after nociceptive threshold testing was completed, animals were retested in 1 hour and then returned to the animal care facility.

Antisense Oligodeoxynucleotides

Nerve Growth Factor (NGF) is a member of the neutrophin family. In addition to their affects on neuronal cell survival, neutrophins can regulate axonal and dendritic growth and guidance, synaptic structures and connections, neurotransmitter release, long term potentiation (LTP), and synaptic plasticity [69,70]. Different neutrophins show binding specificity for particular receptors, NGF preferentially binds to TrkA. Antisense for the 22-mer TrkA ODN was purchased from Invitrogen (San Francisco, CA). The antisense ODN sequence, 5'-CAT CAA CGA AGT CAC CAG ACC G-3', was directed against a unique sequence of rat TrkA. The corresponding GenBank accession number and ODN position within the cDNA sequence are NM021589 and 121-142, respectively [71]. Prior to being used, ODN was lyopholized, reconstituted in nuclease-free 0.9% NaCl to a concentration of the dose administered in each experiment, and stored at -20°C.

The 20-mer PKCε antisense ODN was purchased from Invitrogen (San Francisco, CA). The antisense ODN sequence, 5'-GCC AGC TCG ATC TTG CGC CC-3', was directed against a unique sequence of rat PKCε. The corresponding GenBank accession number and ODN position within the cDNA sequence are XM345631 and 226-245, respectively [71].

For each injection, rats were anesthetized with 2.5 to 5% isoflurane inhalation anesthetic (in O_2). A 30-gauge needle was inserted intrathecally on the midline between the fourth and fifth lumbar vertebrae. In each experiment a control group received intrathecal normal saline vehicle and underwent identical handling and procedures. All doses of antisense ODN and normal saline were administered in a volume of 20 µl once daily as previously described [72,73] for 5 days prior to and for 4 days post-burn, unless otherwise noted. The animals regained consciousness approximately 1 to 3 minutes after the discontinuation of anesthesia.

Thermal Burn

Immediately after baseline nociceptive thresholds were measured, the rats were anesthetized and a thermal burn was induced. For this procedure the rat was placed in an anesthesia induction box with isoflurane 2.5 to 5% delivered in a 50:50 mixture of oxygen and room air. With the loss of spontaneous movement, lack of response to a firm pinch on the forepaw, and lack of a corneal reflex, the animal was removed from the induction chamber and anesthesia was maintained with isoflurane delivered by nose cone. Briefly, the plantar surface of the heel of the right hind paw was placed on a hot plate with a surface temperature of 52.5±0.1°C. A 9-gram sand pouch was placed on the dorsum of the hind paw to maintain gentle, equal pressure to the heel area on the hot plate. Exposure to the thermal stimulus was maintained for 45 seconds. After this, the animal was placed supine on a bed of wood shavings in its housing cage to recover. Spontaneous activity was typically observed within 3 min of removal from anesthesia. Animals were then removed from their housing

cages and placed in the acrylic testing cubicles and allowed to adapt prior to nociceptive threshold testing (i.e., HPW) with the EvF. Nociceptive threshold measurements were obtained at 0.5, 1, 2, 3, 4, 6, 8 hours, and 1, 2, 3, 4, 5, 6, and 7 days (i.e., 24, 48, 72, 96, 120, 144, and 168 hrs) following burn injury.

Statistical Analyses

Nociceptive thresholds were determined by averaging 4 EvF measurements assessed at 6 min intervals for each time point, alternating between the right and left hind paws. Nociceptive thresholds were measured in grams (g) ± standard error of the mean (SEM). To facilitate group comparisons, differences in the injured and uninjured group mean nociceptive thresholds were converted to percent change from baseline, with baseline representing 0% on a 0 to 100% scale. A repeated measures analysis of variance (ANOVA) with one within subjects' factor (i.e., time with 14 levels) and one between subjects' factor (i.e., group) was used to determine if there were differences over time in HPW (i.e., mean hind paw withdrawal) between groups. This analysis allows for testing the main effect of time, the main effect of group, and the group x time interaction. The main effect of time indicates if there was a significant difference, over time, in HPW with time collapsed across groups. The main effect of group indicates if there was a significant difference in HPW between the groups averaged across all time points. The group x time interaction indicates if the differences between the groups in HPW depended on time. Post hoc contrasts were used to evaluate differences in nociceptive thresholds at each time point as compared to baseline. A P-value of <0.05 was considered statistically significant. If the group x time interaction was significant, post hoc contrasts were used to examine for differences between groups at each time point. For all of the contrasts, a Bonferroni correction was made to the alpha level,

which was set at P <0.003 (i.e., 0.05 overall alpha ÷ 14 nociceptive threshold assessment time points). These data were analyzed using SPSS®.

Results

Local erythema and edema were noted in the injured paw within 5 minutes following burn injury in a few animals, but there was no change in skin integrity and no blister formation. The erythema and edema were transient, lasting for up to 48 hours. All animals appeared to bear weight normally on both the injured and uninjured paws at all times. They also exhibited exploratory and grooming behavior, gained weight, and never guarded the injured paw except occasionally when the paw was touched during testing.

Nerve Growth Factor Mediates Thermal Burn-Induced Hyperalgesia via TrkA

In the first series of experiments, 3 doses of TrkA antisense ODN were administered to 3 experimental groups (n=4) as follows: 1-a) a low dose, 40 μg; 1-b) a middle dose, 80 μg; and 1-c) the highest dose, 160 μg. These groups were compared to corresponding control groups (n=4). Data were collected for 7 days post-burn in all except experiment 1-a (3 days). However, these data are included because they demonstrate that changes in nociceptive threshold were dependent on the dose of TrkA antisense ODN administered.

Experiment 1-a: TrkA antisense ODN 40 μg vs. normal saline

TrkA antisense ODN 40 μg was administered for 5 days prior to and for 4 days post-burn injury. The ANOVA revealed a significant main effect of time ($F[1,6]=149.998$, $P<0.0001$), but no significant main effect of group, or group x time interaction (Fig. 1).

Experiment 1-b: TrkA antisense ODN 80 μg vs. normal saline

TrkA antisense ODN 80 μg was administered for 5 days prior to and for 4 days post-burn injury. The ANOVA revealed a significant main effect of time ($F[1,6]=3324.494$, $P<0.0001$) and group ($F[1,6]=12.972$, $P=0.011$), but no group x time interaction. A 95% confidence interval indicated that the normal saline group had at least 3% or as much as 14% more primary mechanical hyperalgesia than the group receiving TrkA antisense ODN 80 μg (Fig. 2).

Experiment 1-c: TrkA antisense ODN 160 μg vs. normal saline

TrkA antisense ODN 160 μg was administered for 5 days prior to and for 4 days post-thermal injury. The ANOVA revealed a significant a significant group x time interaction ($F[13,78]=3.981$, $P=0.028$), as well as a significant main effect of time ($F[1,6]=468.981$, $P<0.0001$) and group ($F[1,6]=15.432$, $P=0.008$). A 95% confidence interval indicated that the control group had at least 8% or as much as 35% more primary mechanical hyperalgesia than the group receiving TrkA antisense ODN 160 μg (Fig. 3).

The significant group x time interaction indicated that the differences in percent change in nociceptive threshold between the groups depended on the specific time point at which nociceptive thresholds were compared. Post hoc tests of simple effects indicated that these differences were significant at the first time point, 0.5 hours ($P=0.023$), as well as 1 hour ($P=0.033$), 2 hours ($P=0.039$), 3 hours ($P=0.024$), 4 hours ($P=0.009$), 4 days ($P=0.006$) and 7 days ($P=0.011$) following burn injury.

Protein Kinase C-epsilon Mediates Thermal Burn-Induced Hyperalgesia

In the second series of experiments, 3 doses of PKCε antisense ODN were administered to 3 experimental groups (n=4) as follows: 1-a) a low dose, 40 μg; 1-b) a middle dose, 80 μg; and 1-c) the highest dose, 160 μg. These groups were compared to corresponding control groups (n=4). Data were collected for 7 days post-burn.

Experiment 2-a: PKCε antisense ODN 40 μg vs. normal saline

PKCε antisense ODN 40 μg was administered for 5 days prior to and for 4 days post-burn. The ANOVA revealed a significant main effect of time ($F[1,6]=326.075$, $P<0.0001$), but no significant main effect of group, or group x time interaction (Fig. 4).

Experiment 2-b: PKCε antisense ODN 80 μg vs. normal saline

PKCε antisense ODN 80 μg was administered for 5 days prior to and for 4 days post-burn injury. The ANOVA revealed a significant main effect of time ($F[1,6]=738.300$, $P<0.0001$) and group ($F[1,6]=22.434$, $P=0.033$), but no group x time interaction. A 95% confidence interval indicated that the normal saline group had at least 9% or as much as 29% more primary mechanical hyperalgesia than the group receiving PKCε antisense ODN 80 μg (Fig. 5).

Experiment 2-c: PKCε antisense ODN 160 μg vs. normal saline

PKCε antisense ODN 160 μg was administered for 5 days prior to and for 4 days post-thermal injury. The ANOVA revealed a significant main effect of time ($F[1,6]=4588.813$, $P<0.0001$) and group ($F[1,6]=313.056$, $P<0.0001$), but no group x time interaction. A 95% confidence interval indicated that the control group had at least 23% or as much as 30% more primary mechanical hyperalgesia than the group receiving PKCε antisense ODN 160 μg (Fig 6.).

Discussion

Intrathecal administration of TrkA and PKCε antisense ODN resulted in a protective effect against primary mechanical hyperalgesia in our thermal burn model. These results support our hypotheses that NGF and PKCε contribute to burn injury pain. We used *in vivo* antisense ODN to inhibit the expression of TrkA and PKCε molecules in primary afferent neurons, as previously confirmed by Western blot analysis [72,74,75]. The exact mechanism

by which antisense ODN inhibit protein expression remains unknown. One theory is that the ODN enter the cell nucleus and bind to receptor channel RNA resulting in the inhibition of gene expression (For review [76]).

TrkA is the high affinity binding site for NGF, a key mediator in the inflammatory process [77]. Thus, we decided to test the hypothesis that receptor TrkA mediate hyperalgesia following a thermal burn. Rats receiving TrkA antisense ODN showed less hyperalgesia compared to control animals receiving normal saline vehicle. These results indicate that the difference between the groups depended on the time that the nociceptive thresholds were measured and the dose of TrkA antisense ODN that was administered, with the highest dose being the most protective.

There was no significant difference between the group receiving TrkA antisense ODN 40 μg and the control group. Doubling the dose to 80 μg resulted in a significant, albeit small, mean 9 ± 2% difference between the groups (Fig. 7). Redoubling the dose to 160 μg more than doubled the effect, resulting in a mean 22 ± 4% more primary mechanical hyperalgesia in the control group than in the group receiving TrkA antisense ODN. These results indicate that NGF contributes to thermal burn-induced primary mechanical hyperalgesia.

These data are consistent with reports of increased levels of NGF in local tissue in animal (rat) models of inflammatory pain [78,79]. For example, following intraplantar injection of C Freund's adjuvant, NGF levels in inflamed tissue were found to be 150 to 200% higher than in noninflamed control tissue at the first assessment time point, 1 day following injection, as measured by 2-site ELISA assay [79]. Specifically following burn injury, however, studies that examined levels of NGF in local tissue are difficult to find. One study examined a wide array of gene expression following a full-thickness 30% total body surface area (TBSA) burn injury in rats [44]. In burned tissue, genes for NGF-induced Factor

A (Gene Bank Accession #AF023087 and #M18416) increased 12- and 15-fold over non-burned control tissue at the first assessment time point, 2 hours following injury. These levels had increased further at the next assessment time point, 6 hours, following thermal injury by 15- and 21-fold. These data are also consistent with Matsuda et al. [43] who found elevated levels of NGF in burn wound edges in a murine model at 2 time points, 6 hours and 3 days, following thermal injury (4.67 ng/g ± 0.11; these values represent the mean ± SEM of 5 separate experiments in duplicate).

In another study, Ueda et al. [45] found NGF was implicated in the development of systemic (i.e., remote) hyperalgesia following a 25% TBSA full-thickness thoracic scald burn in rats [80]. Behavioral testing, using von Frey filaments, revealed that mechanical hyperalgesia developed in the neck, flank, and hind paws beginning 5 days and lasting 3 weeks, with the most intense hyperalgesia detected 2 weeks following injury. They found that the concentration of NGF in the healing tissue increased markedly in these animals (1.10, 2.57, 2.17, 1.15, and 1.70 pg/mg protein after 3, 7, 14, 21 and 28 days respectively, while those in the sham burn group were 0.40, 0.30, 0.23, 0.16 and 0.12 pg/mg protein). Moreover, rats injected daily with anti-NGF serum did not develop hyperalgesia, suggesting that increased NGF in the tissue of the healing skin was a key factor causing systemic hyperalgesia in this model.

NGF is known to induce hyperalgesia following exogenous administration either into peripheral tissue or systemically [46,81-83]. For example, Lewin et al. [46,83] found that a single injection of 50 to 500 ng of human recombinant NGF into the plantar skin of adult rat hind paw elicited both thermal and mechanical hyperalgesia in two phases. The first phase appeared less than 30 minutes after NGF treatment, while the second took several hours to emerge and persisted for several days. However, mechanical hyperalgesia was only observed in the second phase, while thermal hyperalgesia was present in both phases. Lewin et al.

suggested that the first phase of thermal hyperalgesia was due to peripheral action of NGF, while the second phase of thermal and mechanical hyperalgesia required changes in central processing of nociceptive input. These conclusions were based partly on the timing of the effects and partly on the observation that the early phase of thermal hyperalgesia was inhibited by mast cell degranulation, while the latter phase was inhibited by an NMDA antagonist [83]. These findings, taken together with our own, suggest that NGF has profound effects on the activity and plasticity of sensory neurons and may be an important mediator in the induction and maintenance of burn pain. Thus, inhibiting the activity of NGF following a thermal burn may be an important analgesic therapy in the future.

The middle dose (80 μg) of PKCε antisense ODN produced more than twice the protective effect as the same dose of TrkA antisense ODN, resulting in a mean difference of 19±4% and 9±2% more primary mechanical hyperalgesia, respectively, in the corresponding control groups. In contrast, the highest dose (160 μg) doubled the protective effect of TrkA antisense ODN (mean of 22±4%) and only modestly increased the protective effect of PKCε antisense ODN (mean 27±2%). Thus, these findings suggest that PKCε is an important pain pathway signaling molecule in the induction and maintenance of burn pain. However, while TrkA can signal via the PKCε pathway, the present study was not designed to evaluate the role of PKCε in NGF hyperalgesia, which is debatable [51,63,84-86].

Why the primary mechanical hyperalgesia in our model is more robust is not completely understood. We made two modifications to an established thermal burn model [87], both of which probably contributed to increased sensitivity in detecting primary mechanical hyperalgesia. In this regard, we used a different method of measurement, the electronic von Frey as compared to nylon monofilaments, which allowed for a continuous rather than a graded (i.e., logarithmic intervals) measure of nociceptive threshold. We also

used a more stable temperature for our thermal probe, maintaining our thermal stimulus within one-tenth of a degree (±0.1°C) rather than one degree (±1.0°C) Celsius.

Temperature and duration of exposure are critical factors in determining the severity of a burn. For every degree rise in temperature between 41°C and 51°C, the time required to produce epidermal necrosis is reduced by approximately 50% [88]. Therefore, even a small difference in temperature between the models will affect the severity of burn. Thus, it is possible that we induced a deeper dermal burn, which may have sensitized nociceptors in deeper tissues, resulting in more profound primary mechanical hyperalgesia observed in our model.

Conclusion

In conclusion, our thermal burn-induced hyperalgesia model provides a method for elucidating the mechanism and for screening analgesic treatment of this important clinical pain condition. Using our model, the findings from this study indicate that NGF and PKCε contribute to thermal burn-induced primary mechanical hyperalgesia. While NGF has been associated with mechanical hyperalgesia following thermal injury, this is the first report that identifies the epsilon isoform of PKC as a burn pain pathway signaling molecule. This is important because very little is known about the underlying mechanisms of burn pain. Moreover, current analgesic therapies are inadequate to treat burn injury pain, particularly procedural pain. Knowledge of the mechanisms that contribute to burn pain is critical to the development of novel analgesic therapies desperately needed by this vulnerable patient population.

Acknowledgements

The authors would like to thank Dr. Linda Sorkin, Department of Anesthesiology, UCSD, for helping us set up the thermal stimulation device and Drs. Steve Paul and Robert Gear, UCSF, for many helpful discussions and assistance with the statistical analyses. This work was supported by NIH grant #5 F31 NR07474 (GJS) and RO1-DE08973 (JDL).

References

1. Chapman, C.R., Turner, J.A., *Psychological control of acute pain in medical settings.* Journal of Pain Symptom Management, 1986. **1**: p. 9-20.
2. Choinière, M., Melzack, R., Girard, N., Rondeau, J., Paquin, M. J., *Comparisons between patients' and nurses' ratings of pain and medication efficacy in severe burn injuries.* Pain, 1990. **40**: p. 143-152.
3. Difede, J., et al., *Determinants of pain expression in hospitalized burn patients.* Pain, 1997. **72**(1-2): p. 245-51.
4. Geisser, M.E., Bingham, H. G., Robinson, M. E., *Pain and anxiety during burn dressing changes: concordance between patients' and nurses' ratings and relation to medication administration and patient variables.* Journal of Burn Care and Rehabilitation, 1995. **16**: p. 165-171.
5. Gilboa, D., Friedman, M, & Tsur, H, *The burn as a continuous traumatic stress: Implications for emotional treatment during hospitalization.* Journal of Burn Care and Rehabilitation, 1994. **15**: p. 86-91.
6. Malenfant, A., et al., *Tactile, thermal and pain sensibility in burned patients with and without chronic pain and paresthesia problems.* Pain, 1998. **77**(3): p. 241-51.
7. Patterson, D.R., et al., *Lorazepam as an adjunct to opioid analgesics in the treatment of burn pain.* Pain, 1997. **72**(3): p. 367-74.
8. Perry, S., *Undermedication for pain on a burn unit.* General Hospital Psychiatry, 1984. **6**: p. 308-316.
9. Taal, L.A. and A.W. Faber, *Burn injuries, pain and distress: exploring the role of stress symptomatology.* Burns, 1997. **23**(4): p. 288-90.
10. Ulmer, J.F., *An exploratory study of pain, coping, and depressed mood following burn injury.* J Pain Symptom Manage, 1997. **13**(3): p. 148-57.

11. Latarjet, J. and M. Choinière, *Pain in burn patients.* Burns, 1995. **21**(5): p. 344-8.

12. Choinière, M., *Burn pain: A unique challenge.* Pain--Clinical Updates, 2001(Spring).

13. Latarjet, J., *[The pain from burns].* Pathol Biol (Paris), 2002. **50**(2): p. 127-33.

14. Ulmer, J.F., *Burn pain management: a guideline-based approach.* J Burn Care Rehabil, 1998. **19**(2): p. 151-9.

15. American Burn Association. ***www.ameriburn.org***. 2005 [cited 2005 January]; American Burn Association Home Page].

16. Choinière, M., Melzack, R., Papillon, J., *Pain and paresthesia in patients with healed burns: An exploratory study.* Journal of Pain and Symptom Management, 1991. **6**(7): p. 437-444.

17. Malenfant, A., et al., *Prevalence and characteristics of chronic sensory problems in burn patients.* Pain, 1996. **67**(2-3): p. 493-500.

18. Stella, M., et al., *Sensory endings in skin grafts and scars after extensive burns.* Burns, 1994. **20**(6): p. 491-5.

19. Bauer, K.M., Hardy, P. E., Van Dorsten, B., *Posttraumatic stress disorder in burn populations: A critical review of the literature.* Journal of Burn Care and Rehabilitation, 1998. **19**: p. 230-240.

20. van Loey, N.E., et al., *Predictors of chronic posttraumatic stress symptoms following burn injury: results of a longitudinal study.* J Trauma Stress, 2003. **16**(4): p. 361-9.

21. van Loey, N.E. and M.J. van Son, *Psychopathology and psychological problems in patients with burn scars: epidemiology and management.* Am J Clin Dermatol, 2003. **4**(4): p. 245-72.

22. Saxe, G., et al., *Relationship between acute morphine and the course of PTSD in children with burns.* J Am Acad Child Adolesc Psychiatry, 2001. **40**(8): p. 915-21.

23. Yu, B.H. and J.E. Dimsdale, *Posttraumatic stress disorder in patients with burn injuries.* J Burn Care Rehabil, 1999. **20**(5): p. 426-33; discussion 422-5.

24. Carrougher, G.J., et al., *Comparison of patient satisfaction and self-reports of pain in adult burn-injured patients.* Journal of Burn Care and Rehabilitation, 2003. **24**(1): p. 1-8.

25. Gibbons, J., et al., *Opiate-induced respiratory depression in young pediatric burn patients.* J Burn Care Rehabil, 1998. **19**(3): p. 225-9.

26. Honari, S., et al., *Comparison of pain control medication in three age groups of elderly patients.* J Burn Care Rehabil, 1997. **18**(6): p. 500-4.

27. Patterson, D.R., et al., *The 2002 Lindberg Award. PRN vs regularly scheduled opioid analgesics in pediatric burn patients.* J Burn Care Rehabil, 2002. **23**(6): p. 424-30.

28. Sullivan, S.R., et al., *"Opioid creep" is real and may be the cause of "fluid creep".* Burns, 2004. **30**(6): p. 583-90.

29. Bessou, P. and E.R. Perl, *Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli.* Journal of Neurophysiology, 1969. **32**: p. 1025-1043.

30. Campbell, J.N. and R.A. Meyer, *Sensitization of unmyelinated nociceptive afferents in monkey varies with skin type.* Journal of Neurophysiology, 1983. **49**(1): p. 98-110.

31. Fitzgerald, M. and B. Lynn, *The sensitization of high threshold mechanoreceptors with myelinated axons by repeated heating.* Journal of Physiology (London), 1977. **265**: p. 549-563.

32. Koda, H. and K. Mizumura, *Sensitization to mechanical stimulation by inflammatory mediators and by mild burn in canine visceral nociceptors in vitro.* J Neurophysiol, 2002. **87**(4): p. 2043-51.

33. Campbell, J.N., R.A. Meyer, and R.H. LaMotte, *Sensitization of myelinated nociceptive afferents that innervate monkey hand.* Journal of Neurophysiology, 1979. **42**: p. 1669-1679.

34. Jones, D.L. and L.S. Sorkin, *Systemic gabapentin and S(+)-3-isobutyl-gamma-aminobutyric acid block secondary hyperalgesia.* Brain Res, 1998. **810**(1-2): p. 93-9.

35. LaMotte, R.H., et al., *Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat.* The Journal of Neuroscience, 1982. **2**(6): p. 765-781.

36. Pedersen, J.L. and H. Kehlet, *Hyperalgesia in a human model of acute inflammatory pain: a methodological study.* Pain, 1998. **74**(2-3): p. 139-51.

37. Dahl, J.B., et al., *The effect of pre- versus postinjury infiltration with lidocaine on thermal and mechanical hyperalgesia after heat injury to the skin.* Pain, 1993. **53**(1): p. 43-51.

38. Møiniche, S., J.B. Dahl, and H. Kehlet, *Time course of primary and secondary hyperalgesia after heat injury to the skin.* Br J Anaesth, 1993. **71**(2): p. 201-5.

39. Raja, S.N., J.N. Campbell, and R.A. Meyer, *Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin.* Brain, 1984. **107 (Pt 4)**: p. 1179-88.

40. Torebjörk, H.E., R.H. LaMotte, and C.J. Robinson, *Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: Simultaneous recordings in humans of sensory judgments of pain and evoked responses in nociceptors with C-fibers.* Journal of Neurophysiology, 1984. **51**: p. 325-339.

41. Thalhammer, J. and R. LaMotte, *Spatial properties of nociceptor sensitization following heat injury of the skin.* Brain Research, 1982. **231**: p. 257-265.

42. Meyer, R.A. and J.N. Campbell, *Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand.* Science, 1981. **213**(4515): p. 1527-9.

43. Matsuda, H., et al., *Role of nerve growth factor in cutaneous wound healing: accelerating effects in normal and healing-impaired diabetic mice.* J Exp Med, 1998. **187**(3): p. 297-306.

44. Spies, M., et al., *Gene expression analysis in burn wounds of rats.* American Journal of Regul Integr Comp Physiol, 2002. **283**: p. R918-R930.

45. Ueda, M., et al., *Nerve growth factor induces systemic hyperalgesia after thoracic burn injury in the rat.* Neurosci Lett, 2002. **328**(2): p. 97-100.

46. Lewin, G.R., A.M. Ritter, and L.M. Mendell, *Nerve growth factor-induced hyperalgesia in the neonatal and adult rat.* J Neurosci, 1993. **13**(5): p. 2136-48.

47. Lewin, G.R. and L.M. Mendell, *Nerve growth factor and nociception.* Trends Neurosci, 1993. **16**(9): p. 353-9.

48. McMahon, S.B., *NGF as a mediator of inflammatory pain.* Philos Trans R Soc Lond B Biol Sci, 1996. **351**(1338): p. 431-40.

49. Woolf, C.J., *Phenotypic modification of primary sensory neurons: The role of nerve growth factor in the production of persistent pain.* Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 1996. **351**: p. 441-448.

50. Fukumoto, S., et al., *GD3 synthase gene expression in PC12 cells results in the continuous activation of TrkA and ERK1/2 and enhanced proliferation.* J Biol Chem, 2000. **275**(8): p. 5832-8.

51. Gil, C., I. Chaib-Oukadour, and J. Aguilera, *C-terminal fragment of tetanus toxin heavy chain activates Akt and MEK/ERK signalling pathways in a Trk receptor-dependent manner in cultured cortical neurons.* Biochem J, 2003. **373**(Pt 2): p. 613-20.

52. Parran, D.K., S. Barone, Jr., and W.R. Mundy, *Methylmercury inhibits TrkA signaling through the ERK1/2 cascade after NGF stimulation of PC12 cells.* Brain Res Dev Brain Res, 2004. **149**(1): p. 53-61.

53. Soltoff, S.P., et al., *Nerve growth factor promotes the activation of phosphatidylinositol 3-kinase and its association with the trk tyrosine kinase.* J Biol Chem, 1992. **267**(24): p. 17472-7.

54. Raffioni, S. and R.A. Bradshaw, *Activation of phosphatidylinositol 3-kinase by epidermal growth factor, basic fibroblast growth factor, and nerve growth factor in PC12 pheochromocytoma cells.* Proc Natl Acad Sci U S A, 1992. **89**(19): p. 9121-5.

55. Obermeier, A., et al., *Identification of Trk binding sites for SHC and phosphatidylinositol 3'-kinase and formation of a multimeric signaling complex.* J Biol Chem, 1993. **268**(31): p. 22963-6.

56. Vetter, M.L., et al., *Nerve growth factor rapidly stimulates tyrosine phosphorylation of phospholipase C-gamma 1 by a kinase activity associated with the product of the trk protooncogene.* Proc Natl Acad Sci U S A, 1991. **88**(13): p. 5650-4.

57. Obermeier, A., et al., *Tyrosine 785 is a major determinant of Trk--substrate interaction.* Embo J, 1993. **12**(3): p. 933-41.

58. Hundle, B., et al., *Overexpression of epsilon-protein kinase C enhances nerve growth factor-induced phosphorylation of mitogen-activated protein kinases and neurite outgrowth.* J Biol Chem, 1995. **270**(50): p. 30134-40.

59. Hundle, B., et al., *An inhibitory fragment derived from protein kinase Cepsilon prevents enhancement of nerve growth factor responses by ethanol and phorbol esters.* J Biol Chem, 1997. **272**(23): p. 15028-35.

60. Zhou, Y., G.D. Li, and Z.Q. Zhao, *State-dependent phosphorylation of epsilon-isozyme of protein kinase C in adult rat dorsal root ganglia after inflammation and nerve injury.* J Neurochem, 2003. **85**(3): p. 571-80.

61. Cesare, P., et al., *Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat.* Neuron, 1999. **23**(3): p. 617-24.

62. Ohmichi, M., G. Zhu, and A.R. Saltiel, *Nerve growth factor activates calcium-insensitive protein kinase C-epsilon in PC-12 rat pheochromocytoma cells.* Biochem J, 1993. **295 (Pt 3)**: p. 767-72.

63. Khasar, S.G., et al., *A novel nociceptor signaling pathway revealed in protein kinase C epsilon mutant mice.* Neuron, 1999. **24**(1): p. 253-60.

64. Möller, K.Ä., B. Johansson, and O.-G. Berge, *Assessing mechanical allodynia in the rat paw with a new electronic algometer.* Journal of Neuroscience Methods, 1998. **84**: p. 41-47.

65. Parada, C.A., et al., *Tumor necrosis factor receptor type-1 in sensory neurons contributes to induction of chronic enhancement of inflammatory hyperalgesia in rat.* Eur J Neurosci, 2003. **17**(9): p. 1847-52.

66. Cunha, T.M., et al., *An electronic pressure-meter nociception paw test for mice.* Braz J Med Biol Res, 2004. **37**(3): p. 401-7.

67. Vivancos, G.G., et al., *An electronic pressure-meter nociception paw test for rats.* Braz J Med Biol Res, 2004. **37**(3): p. 391-9.

68. Taiwo, Y.O., T.J. Coderre, and J.D. Levine, *The contribution of training to sensitivity in the nociceptive paw-withdrawal test.* Brain Research, 1989. **487**(1): p. 148-151.

69. McAllister, A., L. Katz, and D. Lo, *Neutrophins and synaptic plasticity.* Annual Review of Neuroscience, 1999. **22**: p. 295-318.

70. Poo, M.-M., *Neutrophins as synpatic modulators.* Nature Review Neuroscience, 2001. **2**: p. 24-31.

71. Altschul, S.F., et al., *Gapped BLAST and PSI-BLAST: A new generation of protein database search programs.* Nucleic Acids Research, 1997. **25**: p. 3389-3402.

72. Dina, O.A., Parada, C. A., Yeh, J., Chen, X., McCarter, G. C. et al., *Integrin signalling in inflammatory and neuropathic pain in the rat.* European Journal of Neuroscience, 2004. **19**: p. 634-642.

73. Papir-Kricheli, D., et al., *Behavioural effects of receptor-specific substance P agonists.* Pain, 1987. **31**(2): p. 263-76.

74. Khasar, S.G., M.S. Gold, and J.D. Levine, *A tetrodotoxin-resistant sodium current mediates inflammatory pain in the rat.* Neurosci Lett, 1998. **256**(1): p. 17-20.

75. Alessandri-Haber, N., et al., *Transient receptor potential vanilloid 4 is essential in chemotherapy-induced neuropathic pain in the rat.* Journal of Neuroscience, 2004. **24**(18): p. 4444-52.

76. Crooke, S.T., *Antisense Strategies.* Current Molecular Medicine, 2004. **4**: p. 465-487.

77. McMahon, S.B. and D.L. Bennet, *Trophic factors and pain*, in *Textbook of Pain*, P.D. Wall and R. Melzack, Editors. 1999, Churchill Livingstone: New York. p. 105-128.

78. Bennett, D.L., et al., *Nerve growth factor and sensory nerve function*, in *Pain and Neurogenic Inflammation*, S.D. Brain and P.K. Moore, Editors. 1999, Birkhäuser Verlag: Boston. p. 167-193.

79. Amaya, F., et al., *NGF and GDNF differentially regulate TRPV1 expression that contributes to development of inflammatory thermal hyperalgesia.* European Journal of Neuroscience, 2004. **20**: p. 2303-2310.

80. Osgood, P.F., Murphy, J. L., Carr, D. B., Szyfelbein, S. K., *Increases in plasma beta-endorphin and tail flick latency in the rat following burn injury.* Life Sciences, 1987. **40**: p. 547-554.

81. Andreev, N., et al., *Peripheral administration of nerve growth factor in the adult rat produces a thermal hyperalgesia that requires the presence of sympathetic post-ganglionic neurones.* Pain, 1995. **63**(1): p. 109-15.

82. Davis, B.M., et al., *Altered expression of nerve growth factor in the skin of transgenic mice leads to changes in response to mechanical stimuli.* Neuroscience, 1993. **56**(4): p. 789-92.

83. Lewin, G.R., A. Rueff, and L.M. Mendell, *Peripheral and central mechanisms of NGF-induced hyperalgesia.* European Journal of Neuroscience, 1994. **6**(12): p. 1903-12.

84. Shu, X. and L.M. Mendell, *Acute sensitization by NGF of the response of small-diameter sensory neurons to capsaicin.* J Neurophysiol, 2001. **86**(6): p. 2931-8.

85. Bonnington, J.K. and P.A. McNaughton, *Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor.* J Physiol, 2003. **551**(Pt 2): p. 433-46.

86. Malik-Hall, M., O.A. Dina, and J.D. Levine, *Primary afferent nociceptor mechanisms mediating NGF-induced mechanical hyperalgesia.* Eur J Neurosci, 2005. **21**(12): p. 3387-94.

87. Nozaki-Taguchi, N. and T.L. Yaksh, *A novel model of primary and secondary hyperalgesia after mild thermal injury in the rat.* Neurosci Lett, 1998. **254**(1): p. 25-8.

88. Moritz, A.R. and F.C. Henriques, *Studies of thermal injury, II. The relative importance of time and surface temperature in the causation of cutaneous burns. An experimental study.* American Journal of Pathology, 1947. **23**: p. 695-720.

Figure Legends

Fig 1. Group 1 (closed circles) received intrathecal (IT) TrkA antisense ODN (AS) 40 μg in 20 μl daily for 5 days prior to and for 3 days post-thermal burn (n=4); Group 2 (open circles) received IT normal saline in the same volume over the same period of time (n=4). There was a significant difference over time in nociceptive thresholds, with time collapsed across the groups ($F[1,6]=149.998$, $P=<0.0001$).

Fig 2. Group 1 (closed circles) received intrathecal (IT) TrkA antisense ODN (AS) 80 μg in 20 μl daily for 5 days prior to and for 4 days post-thermal burn (n=4); Group 2 (open circles) received IT normal saline in the same volume over the same period of time (n=4). There was a significant difference over time in nociceptive thresholds, with time collapsed across the groups ($F[1,6]=3324.494$, $P=<0.0001$). There was also a significant difference in nociceptive thresholds between the groups averaged across all time points ($F[1,6]=12.972$, $P=0.011$).

Fig 3. Group 1 (closed circles) received intrathecal (IT) TrkA antisense ODN (AS) 160 μg in 20 μl daily for 5 days prior to and for 4 days post-thermal burn (n=4); Group 2 (open circles) received IT normal saline (NS) in the same volume over the same period of time. There was a significant time x group interaction that indicated the differences between the groups in nociceptive thresholds depended on the time that the nociceptive thresholds were measured ($F[13,78]=3.98.1$, $P=0.028$). A 95% CI indicated that the NS group had at least 8% or as much as 35% more primary mechanical hyperalgesia than the group receiving TrkA AS. There was also a significant difference over time in nociceptive thresholds, with time collapsed across the groups ($F[1,6]=468.981$, $P=<0.0001$), as well as a significant difference

in nociceptive thresholds between the groups averaged across all time points (F[1,6]=15.432, P=0.008).

Fig 4. Group 1 (closed circles) received intathecal (IT) PKCε antisense ODN (AS) 40 μg in 20 μl daily for 5 days prior to and for 4 days post-thermal burn (n=4); Group 2 (open circles) received IT normal saline (NS) in the same volume over the same period of time (n=4). There was a significant difference over time in nociceptive thresholds, with time collapsed across the groups (F[1,6]=326.075, P=<0.0001).

Fig 5. Group 1 (closed circles) received intrathecal (IT) PKCε antisense ODN (AS) 80 μg in 20 μl daily for 5 days prior to and for 4 days post-thermal injury; Group 2 (open circles) received IT normal saline (NS) in the same volume over the same period of time. There was a significant difference in nociceptive thresholds over time, with time collapsed across the groups (F[1,6]=738.300, P=<0.0001). There was also a significant difference over time in nociceptive thresholds collapsed across the groups (F[1,6]=22.434, P=0.003). A 95% CI indicated that the NS group had more primary mechanical hyperalgesia by at least 9% or as much as 29% more than the group receiving PKCε AS.

Fig. 6. Group 1 (closed circles) received intrathecal (IT) PKCε antisense ODN (AS) 160 μg in 20 μl daily for 5 days prior to and for 4 days post-thermal injury; Group 2 (open circles) received IT normal saline (NS) in the same volume over the same period of time. There was a significant difference over time in nociceptive thresholds, with time collapsed across the groups (F[1,6]=4588.813, P=<0.0001). There was also a significant difference in nociceptive thresholds between the groups averaged across all time points (F[1,6]=313.056 P=<0.0001). A 95% CI indicated that the NS group had more primary mechanical hyperalgesia by at least 23% or as much as 30% more than the group receiving PKCε AS.

Fig 1. Experiment 1-a: TrkA antisense ODN 40 μg vs. normal saline

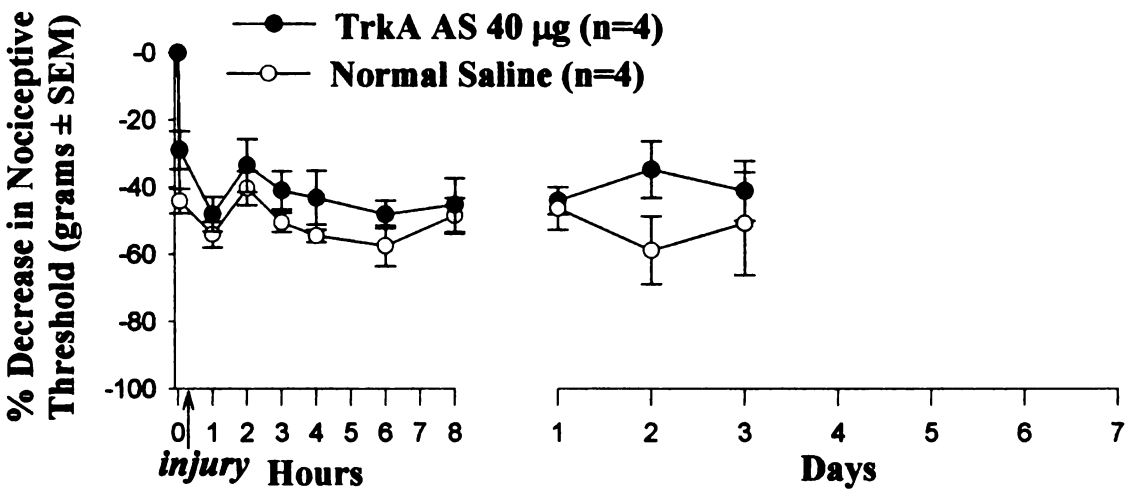


Fig 2. Experiment 1-b: TrkA antisense ODN 80 μg vs. normal saline

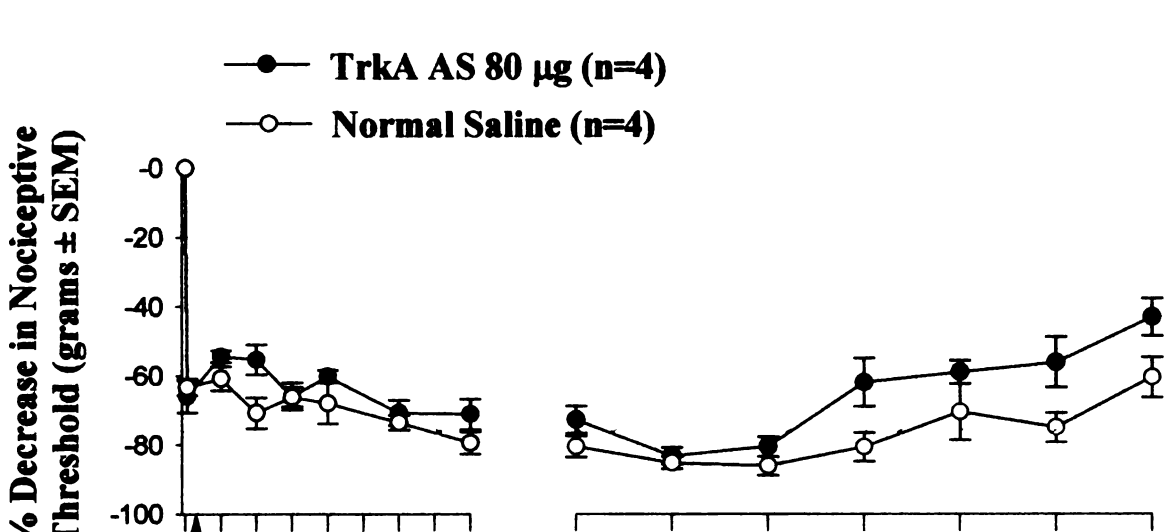


Fig 3. Experiment 1-c: TrkA 160 μg antisense ODN vs. normal saline

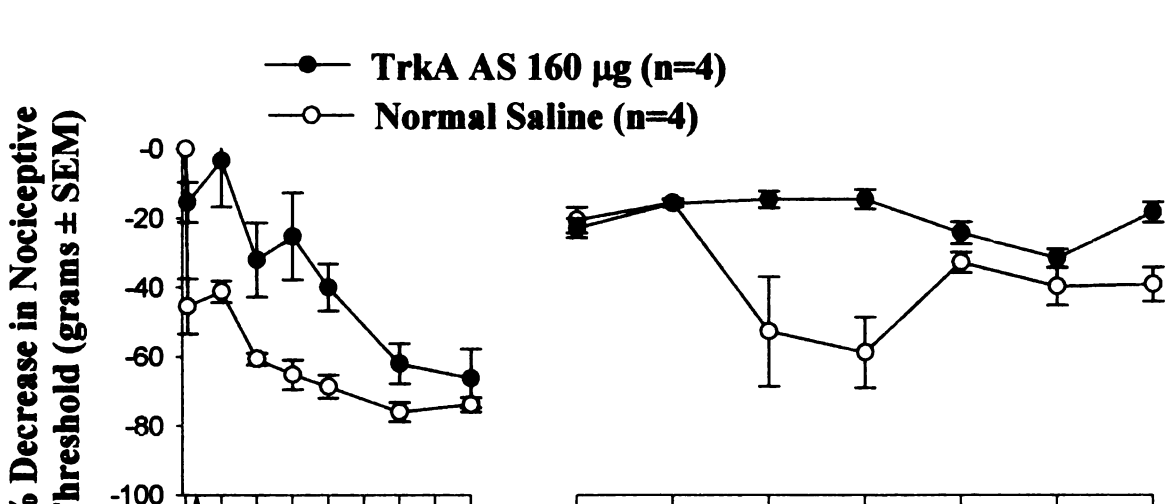


Fig 4. Experiment 2-a: PKCε antisense ODN 40 μg vs. normal saline

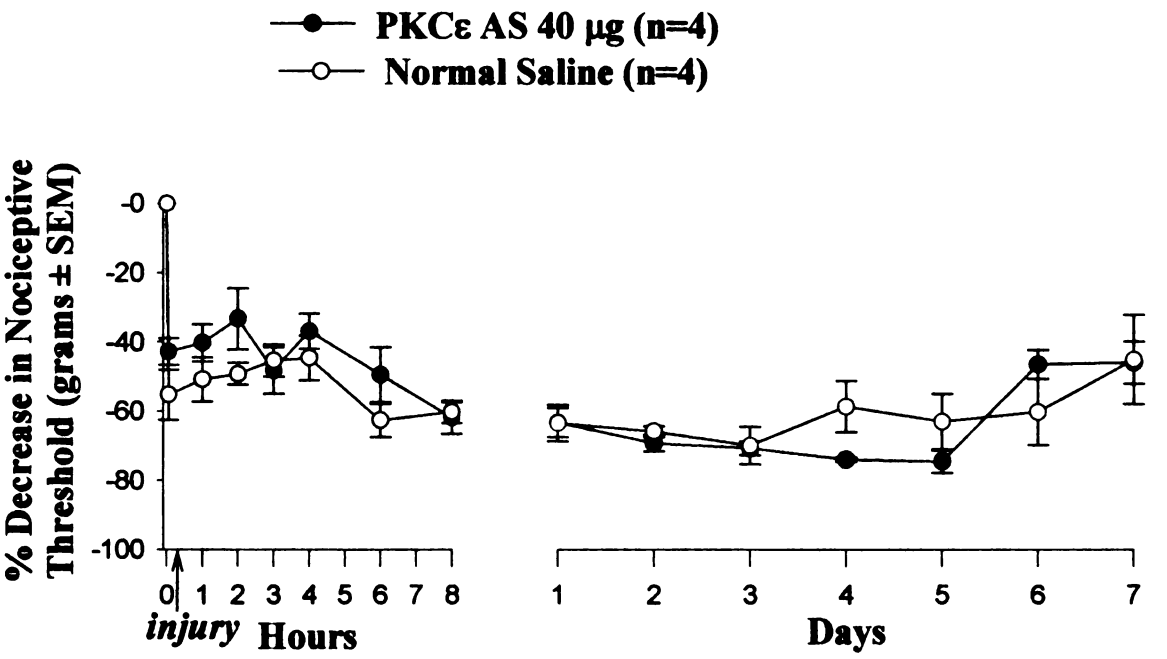


Fig 5. Experiment 2-b: PKCε antisense ODN 80 μg vs. normal saline

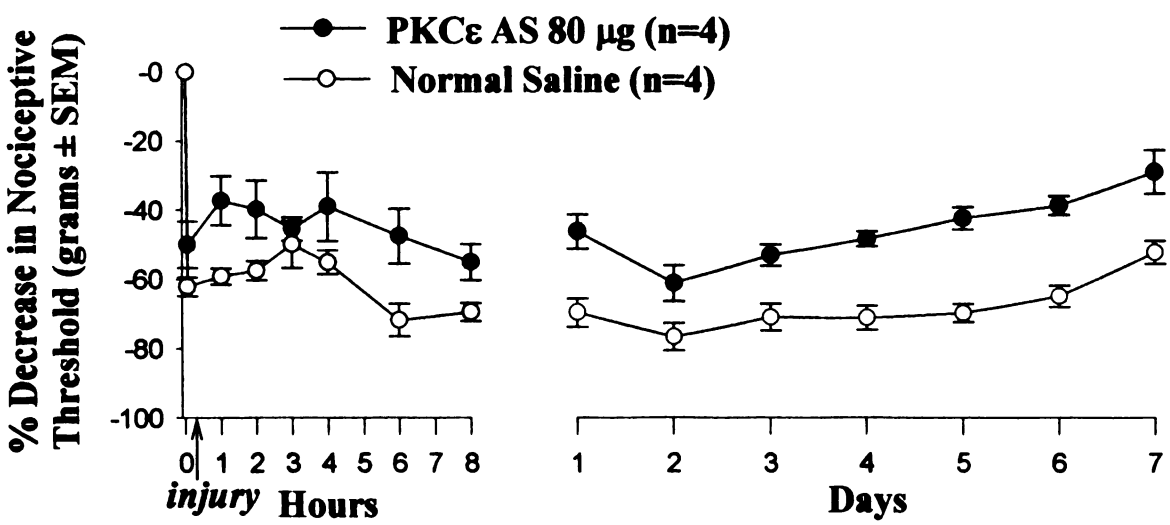
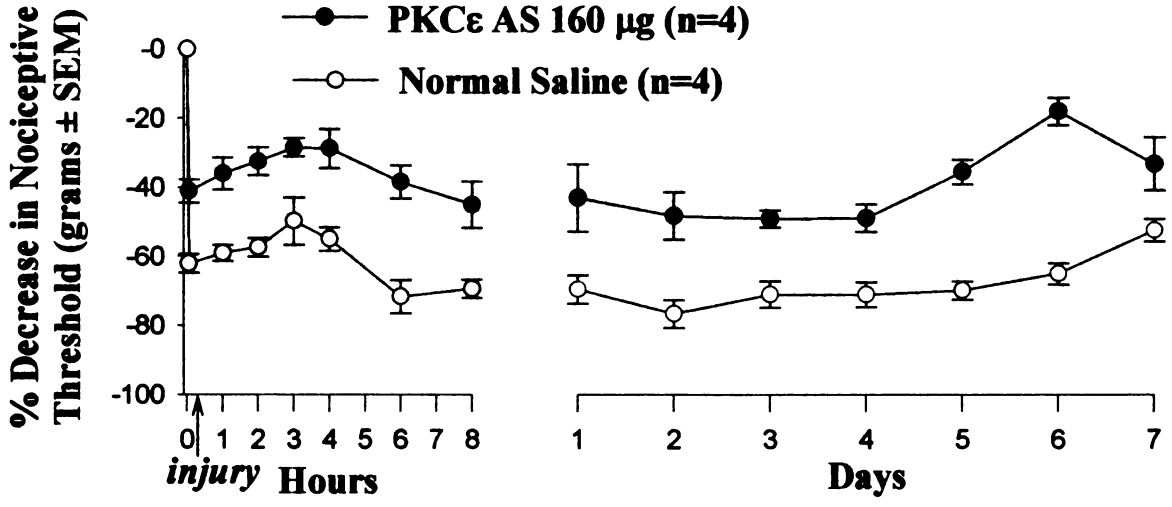


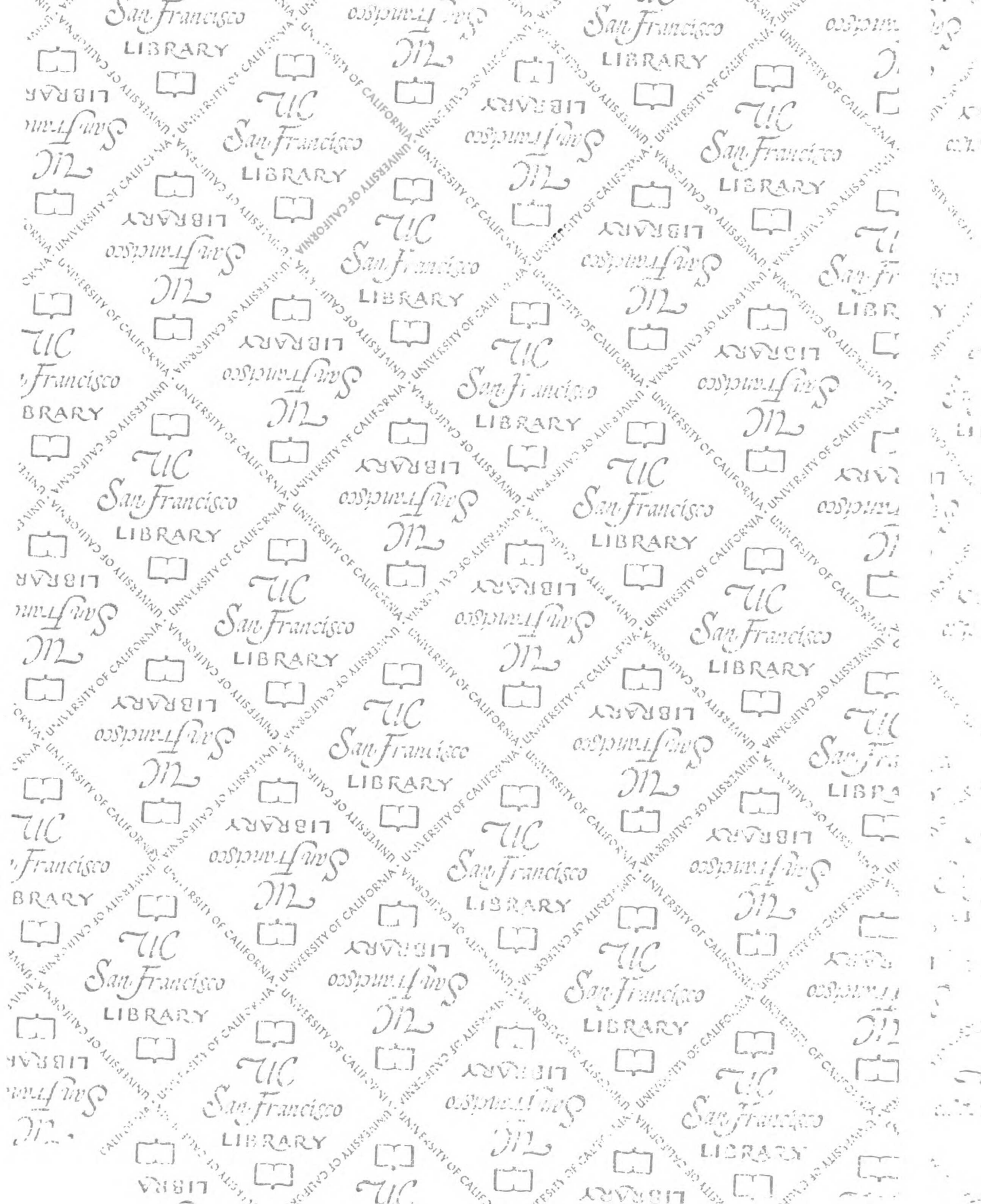
Fig 6. Experiment 2-c: PKCε antisense ODN 160 μg vs. normal saline



Conclusions and Recommendations for Future Research

The purpose of the study presented in Section II of this dissertation was to develop an animal model of thermal burn-induced primary mechanical hyperalgesia that mimics the intensity and duration of clinically observed burn pain. Our model is the first to provide a practical, well-integrated biological system (i.e., *in vivo* method) that demonstrates robust thermal burn-induced primary mechanical hyperalgesia for at least 7 days following injury. Section III describes experiments using this model to elucidate the mechanisms of thermal burn-induced primary mechanical hyperalgesia, the major source of severe pain in burn-injured patients. These data indicate that nerve growth factor (NGF) and protein kinase C-epsilon (PKCε) each may play an important role in burn pain signaling pathways. Moreover, these data demonstrate that the effects of NGF and PKC-ε are dose dependent, suggesting that NGF and PKC-ε inhibiters could prove useful in the treatment of burn pain.

This dissertation provides the foundation for a program of research that will continue to contribute to understanding the mechanisms of thermal burn-induced hyperalgesia. Future research will focus on mechanistic studies to examine the effects of inflammatory mediators on changes in the intensity and duration of acute and neuropathic burn pain. Understanding these mechanisms is important to the discovery of target-specific agents that are critically needed to manage this severe and difficult pain problem. The fact that burn pain remains poorly understood, in part, explains why researchers continue to report that burn pain is poorly managed in most burn centers [5,9-14] and why this program of research is clinically relevant.


UC
San Francisco
LIBRARY
UNIVERSITY OF CALIFORNIA · UNIVERSITY OF CALIFORNIA