Progress of clinical practice on the management of burn-associated pain: What can we learn from animal models?

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Abstract

Opioid-based analgesics provide the mainstay for attenuating burn pain, but they have a myriad of side effects including respiratory depression, nausea, impaired gastrointestinal motility, sedation, dependence, physiologic tolerance, and opioid-induced hyperalgesia. To test and develop novel analgesics, validated burn-relevant animal models of pain are indispensable. Herein we review such animal models, which are mostly limited to rodent models of burn-induced, inflammatory, and neuropathic pain. The latter two are pain syndromes that provide insight into the pain caused by systemic pro-inflammatory cytokines and direct injury to nerves (e.g., after severe burn), respectively. To date, no single animal model optimally mimics the complex pathophysiology and pain that a human burn patient experiences. No currently available burn-pain model examines effects of pharmacological intervention on wound healing. As cornerstones of pain and wound healing, pro-inflammatory mediators may be utilized for insight into both processes. Moreover, common clinical concerns such as systemic inflammatory response syndrome and multiple organ dysfunction remain unaddressed. For development of analgesics, these aberrations can significantly alter the potential efficacy and/or adverse effects of a prescribed analgesic following burn trauma. We therefore suggest that a multi-model strategy would be the most clinically-relevant when evaluating novel analgesics for use in burn patients.

6 Key words: *Burn Pain; Animal Models; Hyperalgesia; Allodynia; Analgesia*

Abbreviations

CFA: Complete Freund's Adjuvant

FTB: Full thickness burn

GFR: Glomerular Filtration Rate

LA: Local Anesthetics

NMDA: N-Methyl_D-aspartic acid

OIH: Opioid-Induced Hyperalgesia

PK: Pharmacokinetics

PD: Pharmacodynamics

PTSD: Post Traumatic Stress Disorder

SIRS: systemic inflammatory response syndrome

SIN: Sciatic Inflammatory Neuritis

SNL: Spinal Nerve Ligation

TBSA: Total Body Surface Area

USAISR: US Army Institute of Surgical Research

Introduction

Burns represent a major public health concern and burden to the healthcare system due to significant morbidity and mortality. The annual cost of the treatment of burn patients in the US is estimated at more than \$573 million [1] and the mean cost for each pediatric patient is \$83,535 [2,3]. However this figure can exceed several hundred thousand dollars and doesn't include outpatient costs such as continued rehabilitation, behavioral health support, or other quality of life indices [4,5]. Inadequate control of severe burn pain, as with other types of trauma, may result in delaying the discharge of burn patients from treatment centers [6,7].

Early and aggressive attenuation of pain is not only important for keeping the patient comfortable throughout the wound healing process, but it is also necessary for increased quality of life years after recovery [8]. Post-Traumatic Stress Disorder (PTSD) may develop in burn patients and can become chronic. In line with this, a retrospective cohort study showed that pain levels were positively correlated with PTSD checklist-military survey scores but not with injury severity scores, suggesting that early acute pain is related to PTSD symptoms [9]. Additionally, other symptoms in the acute setting such as depression, anxiety, and insomnia also appear to affect chronicity of burn pain [10,11]. In fact, the pain experienced by burn victims at discharge is indicative of long-term suicidal ideation [12]. Moreover, in a study examining 358 patients with an average of 12 years between burn and survey response, 52% claimed to have ongoing burn-related pain, 66%

reported that pain interfered with their rehab and 55% felt that it interfered with their daily lives [13].

Therefore, the acute and chronic pain experienced by burn patients remains a significant challenge for patients and clinicians alike, with a demonstrated need for high quality research to guide and improve therapeutic options. Moreover, the pain experienced by burn patients often evolves from time of injury through recovery, and the development of targeted analgesics for each stage of burn care is needed as a means of reducing cost and improving quality of life. Various pre-clinical animal models will prove to be invaluable tools to develop novel analgesics for targeting these different stages of pain. The advantages and disadvantages of modeling pain in animals is discussed below, with an emphasis on gaps and shortcomings concerning burn-associated pain.

Classifications of Burn Pain

Pain experienced during recovery from burn wounds can be broken into four classes; wound (initial injury), procedural, background, and breakthrough pain; wherein each should receive separate assessment in terms of analgesic need. Wound and procedural pain, the most severe types of burn pain, can be elicited by manipulation of affected tissue (e.g., debridement, dressing changes, physical therapy, baths, removal of skin graft staples), and can include the donor site of a skin graft. Preemptive pain management usually consists of short-acting opioid analgesics, but may also include sedatives, anxiolytics (e.g., propofol, benzodiazepines), lidocaine or other non-opioid analgesics, as

delineated in **Table 1** [14–16]. The goal of this treatment regimen is to reduce burn-associated pain, anxiety, and distress typically associated with these types of procedures. However, since pain is a subjective experience, the specific combination of drugs given should be tailored to the individual patient and their unique pain profile.

Background pain describes the persistent pain that can occur throughout the healing process that lasts until the wound is fully re-epithelized. Background pain is characterized by a ‘throbbing’ and ‘burning’ sensation, experienced even while the patient is not moving. Breakthrough pain is pain experienced above the norm of background pain, and is typically associated with movement. Breakthrough pain can be spontaneous and is described as ‘pounding,’ ‘stinging,’ ‘shooting,’ and ‘prickling.’ Breakthrough pain is managed with opioids, and can develop when serum opioid concentrations fall below their therapeutic levels in the absence of an additional stimulus [14–16]. Background is also typically treated with opioids, however is usually controlled with a lower dose opioid with a longer half-life, such as methadone.

In addition to this classification, the pain experienced by burn victims can also be divided into a combination of multiple general types of pain; non-pathologic nociceptive pain, inflammatory pain, and neuropathic pain. Neuropathic pain is caused by a functional disturbance or pathological change to the peripheral nervous system and results in nociceptor sensitization. It is often resistant to treatment (including opioids) and can become chronic, having profound and deleterious effects on a patient’s life [17,18]. Neuropathic pain is typically described as ‘burning, stabbing, shooting, pins and needles,

and electric shock-like sensations' [19]. Among a cohort of 72 patients with average total body surface area (TBSA) burned of 18%, it was found that neuropathic-pain symptoms develop 4.3 months following the injury and persist for 13.1 months. In this group, patients reported significant pain and itching for more than a year following burn injury [20].

Trauma-induced inflammation has a complex biochemical relationship with both neuropathic pain and with inflammatory pain affected by mediators such as prostaglandins, cytokines, oxygen radicals, histamine, and bradykinin. Specifically, IL-6, IL-1 β , and TNF- α are associated with the induction of hypersensitivity reactions in inflammatory pain states, and are drastically upregulated following a burn [21,22]. Other pro-inflammatory cytokines such as platelet derived growth factor (PDGF), TGF β , IL-8 [23], nerve growth factor (NGF) [24], histamine [25], 5-HT [26], prostaglandins [27], and Substance P [28] are also increased following a burn. All of these markers induce a state of peripheral nociceptor sensitization that results in both: an increase in the perception of pain elicited by a noxious stimulus, termed hyperalgesia; and pain evoked by a normally non-noxious stimulus, termed allodynia [29–31]. Burn-induced inflammatory pain results in sensitization to both thermal (noxious) stimuli and mechanical (non-noxious) stimuli [15,22,32,33]. Coincidentally, the inflammatory mediators that induce sensitization also play a major role in wound resolution in a myriad of species, including humans [23,34–36]. These mediators could therefore serve as Rosetta Stones for evaluating pain between different animal models and could have utility in translating these findings not only to the human perception of pain, but also the impact of analgesics on wound healing.

Current Treatments for Burn Pain and the need for Novel Analgesics

Currently, the gold standard for pain treatment is opioids (**table 1**). Weak opioids (tramadol and meperidine) mainly act through μ -opioid agonism, but may have other mechanisms of action [37]. Morphine, the prototypic strong opioid, has been in use since the 19th century and is today one of the most widely used treatments for burn pain. Morphine has been shown to be effective in controlling burn pain, neuropathic pain, and inflammatory pain both in animal models [38–40] and in humans [18,41,42]. Mechanistically, morphine induces analgesia by agonizing the μ -opioid receptor, inducing G-protein linked signaling cascades that lead to a characteristic analgesic state that persists for several hours [41,43].

A variety of other opioid-based options exist for the treatment of burn pain (e.g., piritramide, hydromorphone, oxycodone, methadone, remifentanyl and fentanyl) with varying degrees of potency and side effects. Fentanyl and methadone, along with morphine, are the most commonly utilized analgesics used in burn patients in the military sector [44]. Fentanyl has a 100-fold higher potency than morphine, as well as an increased side effect profile, and has been shown to effectively attenuate neuropathic pain in humans [45]. Methadone, on the other hand, has a 2-3-fold higher potency compared to morphine, and can serve as an alternative if tolerance to morphine develops [15].

Although they can serve as effective analgesics, opioid-based therapy is associated with a myriad of adverse effects. Respiratory depression, bradycardia, constipation, nausea, impaired GI motility, sedation, dependence and physiologic tolerance are the most

common side effects. These are of particular concern in burn patients due to the increased metabolic demand and cardiac contractility during the hypermetabolic recovery period. Moreover, opioid-induced pruritus following a burn can impair wound healing and cause intense discomfort [46,47]. Given the severity of pain and prolonged period of treatment associated with burns, opioid dose escalation is often needed due to the development of tolerance, which can increase the likelihood of addiction [15]. Additionally, opioid-induced hyperalgesia (OIH) may develop wherein opioids induce short on- and offset anti-nociceptive effects coupled with delayed on- and offset pro-nociceptive effects [48,49]. OIH is counterintuitive in that patients receiving opioids can become *more* sensitive to painful stimuli [49–51].

Importantly, opioids have been shown to impair wound healing. Recently, it has been shown that morphine lengthens the time to wound closure in excisional wounds in rats [52–54] and mice by suppressing angiogenesis [55,56]. Burn wounds, however typically involve more severe tissue damage and the ensuing eschar leads to a more robust and sustained level of inflammation [32], which can enhance scar tissue formation [57]. Other studies using a rat ischemia injury model showed opposing results in which opioids accelerated wound healing, thought to be linked to *enhanced* angiogenesis [58]. These conflicting results can be attributed to variability in animal models and highlights the importance of studying morphine's effect on burns.

Non-opioid analgesics that have indications for burn pain treatment include ketamine, lidocaine, and gabapentin. Ketamine, an NMDA antagonist, has emerged both as a means of reducing opioid-related side effects and as an effective analgesic when given as an

adjunct to opioids [48,50]. Beyond its role as an adjunct therapy to opioids, ketamine can also be given alone due to its analgesic (at lower doses) and sedative (at higher doses) properties. This can be useful for burn patients since ketamine-induced sedation reduces the opioid requirement and the corresponding side effects [59,60]. In these patients ketamine has the advantage of not causing hemodynamic and respiratory suppression commonly seen with opioids[61]. As reviewed by McGuinness et al, some clinical studies support the use of ketamine for controlling burn pain and reducing the secondary hyperalgesia compared to the use of opioids alone. However, further clinical studies are needed to evaluate the efficacy of ketamine as analgesic monotherapy for burn pain [62].

Lidocaine, like other local anesthetics (LA's), is a sodium channel blocker that can be used to attenuate procedural pain, however no changes are seen in opioid consumption, anxiety, or level of satisfaction during dressing changes [63]. Gabapentin, a drug originally developed as an anticonvulsant, is now also used to relieve neuropathic pain in addition to anxiety. In burn units, it's often used to reduce anxiety, particularly stemming from anticipation of dressing changes and surgical procedures [64]. Additionally, combination therapy with morphine and gabapentin confers increased analgesia at lower doses than using either drug alone [65], and in burn patients this combination also results in an overall decreased opioid requirement and sensitization [66,67]. A clinical trial also showed that gabapentin reduced mechanical allodynia following a superficial burn but that this effect was only present in uninjured skin [68]. Gabapentin also reduced burn-induced pruritis [69,70] and decreased phantom limb pain following a surgical amputation [71], warranting further investigation into its effectiveness for burn-pain.

Taken together, opioids invite a number of adverse side effects and complications both in the short- and long-term. By contrast, non-opioids do not provide adequate analgesia compared to opioids, thus identifying the need to test novel analgesics. Testing potential therapies for pain relief will undoubtedly rely on animal models of pain pertinent to burns.

Modeling Pain in Animals

Given the complex nature of a burn injury, no *in vitro* assay exists that can adequately model burn pain states, necessitating the use of animal models [72]. Measuring pain in animal models most commonly involves measuring the animal's reaction to a noxious heat stimulus. An enhanced response to heat in injured tissue relative to non-injured tissue is termed thermal hyperalgesia. The most prominent method to evaluate thermal hyperalgesia is known as the Hargreaves method [73], which involves exposing the hindpaw of a rat to a heated stimulus, which will elicit a pain response in the form of hindpaw withdrawal. The time to the reaction is known as the paw withdrawal latency. The longer the withdrawal latency, the lesser the thermal hyperalgesia experienced. This is compared to baseline responses prior to an injury (incision, burn, inflammatory, etc), to gauge injury-induced hyperalgesia. Similarly, the analgesic effect of drugs or other therapies can be measured before and after treatment of the injured animal [74].

Another major type of pain assessment measures mechanical allodynia, which is a response to mechanical stimuli that would be non-painful (non-noxious) under normal conditions. Stimulation of tissue with Von Frey filaments is the gold standard method for

testing mechanical allodynia in both animal models and in human patients, especially those with neuropathic pain [74]. The Von Frey filaments are blunt-ended and range in diameter: the thicker the filament, the greater the force applied. In animal testing, the filaments are applied to the hindpaw until it bends. This is done in an automated, step-wise manner, from low force to high, as the animal stands on a mesh grid. The force that provokes a paw withdrawal is noted. Allodynia is observed when, on average, a thinner filament elicits a withdrawal response over several trials when compared to a control cohort [74,75].

Models of Inflammatory Pain

As discussed earlier, burn pain is the compounded result of inflammatory, nociceptive, and neuropathic pain types. Therefore, to evaluate novel pharmaceuticals for burn-related pain, we can consider animal models for inflammatory and neuropathic pain, in addition to models specifically for burn pain. These pain models are summarized in **Table 2**, and reviewed below.

Several animal models of inflammatory pain have been developed, each with positive and negative features in terms of applicability to burn pain. Most models involve the injection of an inflammatory substance into the hindpaw of a small animal. The most commonly used of these substances are Complete Freund's Adjuvant (CFA) and carrageenan. CFA is a water-in-oil emulsion containing inactivated and dried *Mycobacterium butyricum*, which produces edema, inflammation and persistent pain that appears within 2-6 hours

and lasts 1-2 weeks [78,79][76], resulting in thermal hyperalgesia and mechanical allodynia [93]. Injection of carrageenan, a mixture of sulfated polysaccharides extracted from seaweed, is considered to induce inflammatory pain over a longer time range [74]. Similar to CFA, carrageenan injected into the plantar surface of the hindpaw has been shown to induce thermal and mechanical hyperalgesia lasting from several hours to weeks [76] [81]. Both CFA and carrageenan induce mechanical allodynia and thermal hyperalgesia and therefore may be considered for burn analgesic studies. Less commonly, other compounds such as capsaicin, formalin, mustard oil, zymosan, and bee venom have been used, but their effects are often short-lived. Formalin injection into the paw, for example, is dose-responsive [99] and leads to a hypoalgesic response in the injected region [100]. This phenomenon of a central zone with no sensation, and a peripheral zone of hypersensitivity is similar to what is observed within the zones of coagulation and hyperemia of burns [101]. However, none of these models completely recapitulate burn pathophysiology and inflammation, and thus are not perfect surrogates for burn-pain.

The inflammatory pain associated with burn injury primarily stems from tissue edema and the action of both local and systemic pro-inflammatory cytokines. Although the currently used inflammatory pain animal models also include edema, thermal hyperalgesia, and mechanical allodynia normally observed in burn-related pain, the effects seen in these models are often short-lived and confined to the tissue near the injection site. These models fail to reproduce the systemic inflammation and inflammation-related pain seen in severely burned patients. Care must be taken when

interpreting results from these studies since the systemic inflammation resulting from a large burn persists for far longer than those induced by the agents used in these models. In short, these models fail to recapitulate burn pathophysiology and inflammation, and thus are not perfect surrogates for burn-pain.

Models of Neuropathic Pain

Nerve damage that occurs in severe burns causes neuropathic pain, which results in hyperalgesia and allodynia. The study of peripheral neuropathy presents a challenge in animals, as it requires a difficult surgery to be performed in order to produce abnormal function of a major nerve. The ensuing nerve damage itself may be a potentially confounding factor when examining hindpaw latency. Multiple methods have been explored to examine neuropathic pain while reducing or eliminating the risk of this complication. The spinal nerve ligation (SNL) model is the most widely used for studying neuropathic pain. The SNL procedure involves ligation of the spinal nerves at the L5 and L6 level, distal to their corresponding dorsal root ganglia [84]. L5/L6 SNL produces a hypersensitivity reaction within 24 hours post-surgery. Mechanical allodynia persists at least 10 weeks, while thermal hyperalgesia persists at least 16 weeks. SNL also produces signs of spontaneous pain and is sympathetically mediated [83,84]. Contralateral mechanical allodynia and thermal hyperalgesia has also been noted both in rats and primates [84,102,103]

The Sciatic Inflammatory Neuritis (SIN) model allows for the investigation of the direct effects of inflammation on the formation of neuropathic pain. Under anesthesia, a catheter is implanted around the sciatic nerve, and fed subcutaneously to exit 1cm rostral to the base of the tail. The pro-inflammatory molecule zymogen is injected 4-5 days following the procedure directly onto the sciatic nerve. At high doses (400 μ g) zymogen induces bilateral allodynia, however this response is not coupled with thermal hyperalgesia. Allodynia forms within 3 hours following injection and persists at least 24 hours, however a long-term study is needed in order to determine the full duration of effects [86]. Zymogen SIN is associated with increased IL-1 β , TNF- α , and reactive oxygen species from peri-sciatic immune cells, and mild edema formation around the sciatic nerve [87].

Inflammation is a staple physiologic response to burn injury, which is addressed with the SIN model described above. For example, following CFA, there is an increase in spontaneous activity within myelinated fibers, which is associated with increases in IL-6 and IL-1 β [108,109]. This model has the advantage of partially replicating the systemic pro-inflammatory cytokine release characteristic of burn injury. However, the immunologic response following a burn is far more prolonged than those elicited by the SIN models, and involves both allodynia and hyperalgesia. In short, these procedures also fail to replicate the complex pathophysiology of burn injury, and the ensuing systemic inflammation. As a result, there is a need to develop burn-specific animal models of pain

that also incorporate the prolonged inflammatory response, in addition to both direct and indirect damage to neuronal tissue.

Models of Burn Pain

As early as 1950, researchers began using thermal injuries to describe primary (at the site of injury) and secondary (proximal to the site of injury) hyperalgesia to mechanical and thermal stimuli [110]. More recently, human models for 1st degree burns have been developed and have been shown to evoke primary and secondary allodynia and hyperalgesia [111] but no spontaneous pain [112]. In a twin subject study in women, researchers found that environmental causes significantly affected the skin flare response to mild burn [113]. In this study, the extent of thermal sensitization was not significantly affected by differences in genetic or environmental backgrounds. Other human models of mild burn injury have also been developed using ultraviolet radiation to model sunburn-evoked inflammation and sensitization [114]. These models can be used to evaluate drug efficacy including those of NSAIDs [115]. Although superficial burn pain models have been developed [116,117], all of these clinical studies only lend insight into milder burn injuries. Ethical considerations dictate that a human model for severe and extensive burn injury will never be developed, thus necessitating animal models.

Several animal models of clinically-relevant burn pain have been developed. Early models involve exposing the dorsal skin to scald injury [118], while other more recent models involve contact burns with brass probes at a constant pressure and temperature to

the dorsum or hindpaw [119]. These models induce mechanical allodynia that persists for at least 1 week and thermal hyperalgesia that lasts for 3 weeks following burn. This response is mediated by TrkA and PKC ϵ and induces transient edema [32,33]. Another recently developed model creates partial-thickness burns on the hindpaw by contact with a hollow metal plate through which hot water circulates. This induces edema and thermal hyperalgesia lasting 7 days , and mechanical allodynia lasting 14 days post-burn. This model has revealed the essential role for the sodium channel Nav1.7 in mediating hypersensitivity reactions [88]. Other rodent variants of this model, using warmer water, elicits allodynia lasting at least 4 weeks post-injury [89]. *Chang et al.* have used a hot plate model to show persistent allodynia (including contralateral) for up to 8 weeks [90].

To elucidate the effect of burn depth on pain development, *Wang et al.* utilized a rodent hindpaw immersion model that varies the exposure time to hot water in order to produce superficial, partial-thickness, and full thickness burns (FTBs). In this model, only the FTB elicited thermal hyperalgesia at all time points in a 7-day study. Mechanical allodynia also persisted at least 3 days for all three burn depths, but quickly resolved in the superficial burns. This model has been used to study the rightward shift over time (between 1 and 2 weeks) of the morphine dose-response curve, indicating the development of opioid tolerance due to down-regulation of spinal μ -opioid receptors following a FTB injury [92].

A more recently developed model for FTB by *Fowler et al.*, utilized a focal injury to the hindpaw by a 100°C slanted soldering tip. This procedure evokes an immediate partial

thickness burn that progresses into a FTB within 2 days. As shown by the lack of hyperalgesia at the primary site of focal FTB injury, neuronal damage occurs in this animal model, and pain testing was performed at a secondary site proximal to the burn. Edema and erythema forming within 1 day following the burn takes 4 weeks to resolve. Thermal hyperalgesia and mechanical allodynia developed within 1 day following the injury and lasted for 2 weeks. Neither hypersensitivity reactions were observed in the contralateral paw despite elevated spinal dorsal horn Substance P and CGRP levels [40].

These animal models provide quantitative readouts of burn pain, and are invaluable as a screening tool for examining novel analgesics [120]. However, they fail to replicate the complex pathophysiology seen in burn patients in several respects. First, these models induce short-lived hypersensitivity reactions, whereas the pain experienced by burn victims is prolonged and dynamic. Second, these models only involve small burns of typically less than 1% TBSA, and hence cannot model the pathophysiology of severe burns. Large TBSA burns are becoming increasingly survivable, and involve an altered metabolic state which changes the pharmacokinetics (PK) of analgesics. Finally, to our knowledge no pre-clinical animal model takes into account other biological processes (e.g., wound healing) that greatly affect the pain experienced by a burn victim. A comprehensive model incorporating both pain and wound healing would be advantageous since these two aspects of care are fundamentally intertwined. In the following section, we discuss these considerations for developing such a pre-clinical burn pain model.

Other animal models of burn: hurdles for incorporating pain

Wound Healing

As mentioned above, the relationship between burn pain and wound healing is inseparable. Direct links (e.g., dressing changes, grating operations) and indirect links (e.g., chronic pain preventing rehabilitation, depression [121,122]) confound the analysis of analgesic treatments in humans. Many animal models of burn wound healing have been reviewed elsewhere [72] and are well beyond the scope of this review. The vast majority of these studies do not address pain, and Animal Care and Use Committee guidelines often require that the subjects remain pain-free throughout the duration of the study [123], thus preventing the study of burn-related pain.

As reviewed above, the testing of pain has primarily been performed in small animals such as mice and rats. Burn wound healing in these animals has also been extensively studied. For example, rat models (reviewed in [124]) have been used to highlight the importance of infections [125] and angiogenesis [126]. Burn wound healing models in both rats and mice have been used to examine the importance of nitric oxide signaling pathways [127,128]. However, both rats and mice have distinct differences in cutaneous structure and healing when compared to humans. Rodents have a very dense layer of hair with a shorter hair cycle (3 weeks vs. several years) when compared to humans. Mouse and rat skin lacks sweat glands, and is elastic since it doesn't have a strong attachment to underlying tissues. Moreover, rodents have a structure known as the panniculus carnosus, a thin muscle layer that's only found in the neck of humans. This layer is ubiquitous in

rodents and results in wound healing by contraction, versus wound re-epithelialization in humans. Indeed, researchers have adapted a variety of surgical approaches in rodents to more closely approximate healing in humans [129]. Taken together, rodents allow for a fast turnaround time for wound healing studies, but structural and healing differences must be taken into account when inferring relevance to human wounds.

Of the laboratory animals used in modeling human skin pathologies, the pig is the most common choice due to similarities in structure and healing profiles [130,131]. Histologically both pig and human skin contain sparse hair follicles, similar dermal and epidermal thicknesses and no panniculus carnosus muscle layer. Many burn wound models exist to evaluate wound healing, debridement, and grafting procedures and have been reviewed elsewhere [72]. Studies involving contact burns in swine typically alter temperature and time of contact of the heat source, with a constant pressure. Yorkshire pigs are most often used, unless scarring is the endpoint, in which case Red Duroc pigs are the species of choice [132].

Several porcine models can be tailored to investigate the effect of treatment or debridement/grafting on wound healing (e.g., contraction, re-epithelialization, graft adherence, fibrin deposition, etc.) [130,133,134]. Explanation of the burning mechanism is of the utmost importance, as scalding, flame burns, contact burns and radiation burns, to name a few, have been utilized [135–138]. A common limitation in these models is a degree of burn depth heterogeneity [139,140], which some investigators go to great lengths to minimize [141]. The model used depends intimately on the question asked. For

example, to study the effects of different types of skin coverage (autografts, allografts, synthetic graft materials), burn excision and grafting in the pig has been performed [137,142]. Attempts to minimize the progression of burn wound depth usually utilize deep partial thickness burns [143].

Despite the obvious advantages of using pigs to study burn wound healing, pain testing in larger animals is far more challenging given their evolutionary adaptation to hide pain as a protective behavior. As a result, current pain testing in porcine models is largely descriptive, and involves observing behavior and weight changes after an injury [144]. Largely, methods of pain quantification in large animals are indirect (i.e. mean arterial pressure, lethargy, body temperature, heart rate, serum cortisol, etc.) and have not been validated as true measures of pain. Therefore, since no quantitative methods have been developed for pain-quantification in large animals, pain testing has largely been confined to rats and mice [145]. Development of quantifiable behavioral measures of pain experienced by large animals such as pigs could greatly advance research of the relationship between wound healing and burn-pain.

Burn pathophysiology

While the burn models mentioned above are useful in examining wound healing, they neglect to consider the greatly altered physiological status of the severely burned patient. The pathophysiological state following severe burns involves a grossly altered immune system and hypermetabolism described in detail elsewhere [146–148]. To complicate the situation further, there are often comorbidities with burn, such as infection and inhalation

injuries. In short, no animal model successfully incorporates the pathophysiological manifestations of hypermetabolism and (systemic inflammatory response syndrome) SIRS, and how that influences burn- associated pain.

This altered physiological state also has drastic effects on pharmacokinetic (PK) and pharmacodynamic (PD) profiles of drugs. PK changes in the acute phase are largely due to decreases in distribution and renal clearance and are a function of the degree of inflammation [15,149]. As a result, burn injury can induce changes in analgesic clearance and half-life, thus affecting their analgesic capabilities [150,151]. Additionally, increased renal and hepatic blood flows are observed due to elevated cardiac output during fluid resuscitation, resulting in an increased glomerular filtration rate and creatinine clearance. Although multiple wounds are created in the models of wound healing described above, the TBSA affected is typically less than 15%. To this end, large TBSA burn models for the purposes of studying SIRS exist (although not typically for the study of wound healing) and have been reviewed in detail elsewhere [152]. In rodents, this is usually performed by scalding, which can be used to examine pharmacological intervention, as these animals have low mortality, even after extensive full thickness burns [153–156]. However, not only is skin structure different in rodents compared to humans, the applicability of inflammation in rodents to the human scenario remains controversial [157]. Moreover, it has been shown that large animals have hypermetabolic responses that more closely resemble what is seen in humans [158,159].

Specifically, pigs have similar metabolic features and hepatic responses to burn injury as humans [160,161]. To this end, only a handful of studies are aimed at recapitulating burn-induced SIRS in large animals, and models are lacking [152]. One example uses a 40% TBSA burn to show that liver triglycerides accumulate following thermal injury due to very low-density lipoprotein inhibition, increased triglyceride synthesis, and impaired oxidation [162]. These changes in liver function, and thus drug metabolism, PK, and PD, like those mentioned earlier, need to be taken into account in evaluating novel analgesics.

Of the limited large animal burn-induced SIRS studies available, only two have addressed inflammation as it relates to burn resuscitation [163,164]. Luo et al. highlighted the utility for large animal models of burn-induced SIRS in studying the effects of the urinary trypsin inhibitor, ulinastatin [165]. Another study examined mobilization of mononuclear cells after a 30%TBSA scald burn [166]. Many previous models that involve extensive burn injuries in large animals also incorporate an associated co-morbidity in the form of LPS injection [167–169] or smoke inhalation [170,171]. The addition of these clinically-relevant co-morbidities can be performed to augment/alter the systemic inflammation.

In short, the multi-factorial response to burn injury is difficult to recapitulate in an animal model. A variety of models exist to address SIRS, hypermetabolism, or wound healing, but few have considered the possibility of evaluating burn-associated pain and administration of analgesics. Ethical and practical considerations make it difficult to truly allow for testing of analgesics in these contexts. Rodent models currently exist as a

screening tool for analgesic efficacy. Porcine models currently exist to examine whether these analgesics positively or negatively affect wound healing. However, there is a need for a large animal model of burn injury that can examine how burn-induced changes in metabolism, pharmacokinetics, inflammation, etc. affect burn-pain and related treatments.

Summary and Clinical Applicability

There is a need for novel analgesics that confer potent pain relief without unwanted side effects for the burn patient. A new analgesic should: have equal or better efficacy than morphine for attenuating burn-related hyperalgesia and allodynia; be long-lasting yet easily titratable; reduce the likelihood of chronic pain and PTSD development; have minimal to no cardiorespiratory depressant effects; not potentiate disruptions to homeostasis caused by burn trauma; and promote wound healing. Moreover, given the variability between the different stages of recovery (acute, hypermetabolic, and rehabilitation), selection of the optimal burn analgesic should be adjusted to the stage of recovery of the patient. For example, an analgesic that attenuates inflammatory pain could be useful in the earlier stages of recovery while one that addresses neuropathic pain could be more useful in the long term scenario.

To address the need, several animal models of neuropathic, inflammatory, and burn pain have been developed. Small animal studies have the advantage of providing measurable pain data but fail to incorporate the systemic effects of a burn injury. Determining the

effect of a drug on the disruptions to metabolism and wound healing in addition to the consequences on SIRS is vital for ensuring more acceptable outcomes. Large animal studies provide this needed information but at a much higher cost compared to small animal studies [72]. Each model and model organism has pros and cons that must be evaluated in concert with one another.

Therefore, we propose that a multi-model strategy must be utilized to truly evaluate a novel analgesic's potential for use in human burn patients. Logistically, we would propose that small animal pain studies should be performed to evaluate a drug's analgesic potential followed by pig studies of large burns to examine systemic effects and wound healing. Ethically this would reduce the number of animals used and minimize the amount of pain transferred. Given that no single pain model accurately describes the total clinical etiology of the burn injury, we must utilize several models in order to gain insight into a novel analgesic's potential in the hyper-specialized burn patient population.

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Progress of clinical practice on the management of burn-associated pain: What can we learn from animal models?

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Table 1: Analgesics Commonly used in Burn Patients. Opioids, non-opioids, and non-pharmacological methods can be used alone or in combination for analgesia.

Table 2: Animal Models of Pain Relevant to Burns. The table below describes the most burn-relevant models for inflammatory, neuropathic, and burn pain. CFA: Complete Freund's adjuvant, SNL: Spinal Nerve Ligature, SIN: Sciatic Inflammatory Neuritis

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Weak Opioids	Strong Opioids	Non-Opioids	Non-Pharmacologics
Tramadol Meperidine (Demerol)	Morphine Fentanyl Methadone Hydromorphone Piritramide Oxycodone Remifentanyl	NSAIDs Paracetamol/APAP Lidocaine/LA's Ketamine Gabapentin Benzodiazepines Anxiolytics	Virtual Reality Acupuncture Acupressure Hypnosis

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Type of Pain	Model	Animal	Induction Method	Duration of Hypersensitivity	Reference
Inflammatory	CFA	Rats, Mice	Hindpaw Injection	1-2 Weeks	[76–80]
	Carrageenan	Rats, Mice	Hindpaw Injection	1-3 Weeks	[74,76,81, 82]
Neuropathic	SNL	Rats, Mice	Surgical Ligation	10 Weeks	[83–85]
	SIN	Rats	Surgical Catheter	3-5 Days	[86,87]
Burn	Partial-Thickness Burn models	Rats, Mice	Heated plate or water	1-8 Weeks	[88–91]
	Full-Thickness Burn Models	Rats	Heated water or probe	1-14 days	[40,92].