

Overview

Influence of Pain and Analgesia on Orthopedic and Wound-healing Models in Rats and Mice

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The surgical stress response and resulting physiologic changes can lead to postoperative complications and negatively impact animal welfare. Although appropriate pain management is crucial to reduce the pain and stress response to surgery, analgesic choice can significantly affect bone and wound healing. This review aims to summarize data from rat and mouse studies and to provide recommendations for integrating analgesia into orthopedic and wound healing models in these species. Data from other species, such as humans, rabbits and other rodents, is included, where available. From these data, we conclude that for orthopedic surgical models, opioids, local anesthetics and dissociative agents have minimal impact on fracture healing; cyclooxygenase 2 (COX2) selective nonsteroidal antiinflammatory drugs (NSAID) may be used in the short-term; and steroids should be avoided. For wound healing models, short-term systemic or topical opioids have negligible impact on wound healing; NSAID or local anesthetics may be used short-term; and systemic steroids should be avoided. Alternative analgesics such as tramadol, gabapentin, ketamine, and acetaminophen warrant consideration and further evaluation for both orthopedic and wound healing models. In all cases, researchers and veterinarians should work together to determine the appropriate analgesic plan to minimize pain, as well as to minimize unwanted effects on the orthopedic and wound healing models themselves.

Abbreviations: LA, local anesthetic; cyclooxygenase (COX); PO, *per os*; ACTH, adrenocorticotrophic hormone; TSH, thyroid stimulating hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone.

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Rats and mice as surgical orthopedic and wound healing models. Rodents, including rats (38%) and mice (15%), comprise over half of the animals used in orthopedic research.¹⁰² Rats are commonly used as subjects for orthopedic studies of fracture, bone defect repair, bone ingrowth, bone or joint infections, osteoporosis, osteomyelitis, bone circulation, prosthetic debris, biocompatibility, and nerve repair.⁶ Mice are popular as orthopedic models because of ease of genetic manipulation and are most often used to study osteogenesis, chondrogenesis, osteoporosis, arthritis, bone tumor, and nerve repair.⁶ There are a number of rodent wound healing models: incisional, excisional, dead space, flap surgery, burns, aging, and dermonecrotic models in various locations including the dorsal, ventral, or neck areas, as well as the flank, leg, and feet areas.^{8,39,74,79,119} Based on the assumption that what is painful to a human would be painful to another animal, analgesia should be included as part of the experimental design for all experimental surgical procedures.²⁰

When using animals in research, recognition and treatment of pain are essential for both ethical and regulatory reasons. Justification must be provided to an Institutional Animal Care and Use Committee (IACUC) or similar oversight body to document both the need to perform the particular surgical procedures in live animals and the analgesic measures that will be taken to mitigate pain.²⁰ The US Government *Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and*

Training states that the minimization of pain is imperative, and that procedures must be performed using “appropriate sedation, analgesia or anesthesia”.³⁰ Likewise, the *Guide for the Care and Use of Laboratory Animals* (“The Guide”) states that “The selection of appropriate analgesics and anesthetics should reflect professional veterinary judgment as to which best meets clinical and humane requirements as well as the needs of the research protocol.”^{62,98} In addition to being an ethical and regulatory requirement, improper pain management can also have significant implications on study outcomes and accurate evaluation of biologic effects.^{66,81} However, when providing analgesia for orthopedic and wound healing models, the breadth of analgesia options can complicate devising an analgesic plan that is effective and does not interfere with the study objectives. This challenge is further complicated by scientific literature that inconsistently reports the use of anesthetics and analgesics during experimental procedures.²¹ In this review, we summarize the scientific literature available to provide recommendations for integrating analgesia into rat and mouse orthopedic and wound healing models. When only minimal data is available from rat and mouse studies, we draw from information available from other species including humans, rabbits and other rodents.

Pain and the surgical stress response. Stress has been demonstrated to elicit endogenous pain relief, a phenomenon known as stress-induced analgesia.¹⁸ The endogenous pain inhibitory systems can be activated by fear, anxiety and stress.^{2,5,104} Stress-induced analgesia depends on, intensity and duration of the stress, and the level of control the animal has over its stress.

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This suggests that the context in which the animal perceives the stressor is a major determining factor of the perceived pain.^{5,58,65}

The phrase “surgical stress response” is used to describe the physiologic response (including hormonal and metabolic changes) to surgery and pain.^{26,70} The 3 key components of the surgical stress response are: 1) sympathetic nervous system activation, 2) endocrine response, and 3) immunologic and hematologic changes.^{25,26,33} In brief, the sympathoadrenal response results from increased catecholamine secretion. Prolonged release of circulating norepinephrine and epinephrine leads to tachycardia and hypertension, affecting the function of the liver, pancreas, and kidney. The endocrine response involves changes in pituitary hormone secretion that include increased ACTH, growth hormone, and arginine vasopressin; and increased or decreased TSH, FSH and LH.²⁵ These changes result in secondary effects on hormone secretion in target organs, including the adrenal cortex (increased cortisol and aldosterone), pancreas (decreased insulin and increased glucagon), and thyroid (decreased thyroxine).²⁵ The overall outcome is increased catabolism, mobilization of energy stores, and retention of salt and water to maintain fluid volume and cardiovascular hemostasis.²⁵ Protein catabolism and hyperglycemia are linked to muscle wasting, weight loss, impaired wound healing, and infection.²⁵ The immunologic changes include increased production of cytokines and acute phase reactants, reduced recruitment of inflammatory cells to the wound margin, impaired antibacterial function and slowed healing.^{28,124} A generalized state of immunosuppression following surgery or trauma is implicated in the development of septic complications postoperatively.^{82,89,91} The greater the surgical stress, the more profound the effect on the immune system and healing.⁸² Although the surgical stress response may represent a conserved defense mechanism,⁹² the resulting physiologic changes may lead to postoperative complications and can interfere with orthopedic and wound healing.

Pain associated with surgery and the pain associated with surgical wounds is the consequence of stimulation of nociceptors and the subsequent changes in nociceptor nerve endings (C and A- δ nerve fibers) which are processed by numerous neuronal networks and brain structures.^{12,66} Although pain serves a protective function to warn the body of damage and to seek protection, untreated surgical pain and stress slow healing.⁸⁸ For example, postoperative pain in human patients predicts longer length of hospital stay, delayed ambulation, and long-term functional impairment.⁹⁵ Surgical pain in elderly human patients has been linked to poor quality of life, reduced wellbeing, physical disability and mortality.^{71,88,121} Similarly, untreated pain in rodents may lead to reduced food and water intake, disrupted sleep, and changes in activity and species-specific behaviors, including grooming, nesting and burrowing.^{66-68,83} Without effective analgesia, healing can be delayed. In humans, greater surgical pain is associated with slower healing of experimental wounds.⁸⁸ For orthopedic procedures, effective pain control is vital to promote continued limb usage to stimulate bone healing.^{66,120} In Sprague–Dawley rats, mechanical loading is essential to femoral bone healing.^{120,139,140,141} Without adequate analgesia, continued limb usage and the mechanical stress required for proper healing is hampered.¹²⁰ Analgesia will reduce pain, but not eliminate all painful sensations, therefore, providing analgesia is unlikely to result in overuse injury.

An effective way to reduce the stress and pain response to surgery is to provide preemptive and/or multimodal analgesia. For example, rats treated with morphine 30-min prior to

surgery showed reduced postsurgical increases in plasma corticosterone.¹⁰⁷ Because pain pathways are complex, a multimodal analgesia plan is most likely to offer effective pain management. However, analgesic choice must be carefully considered as the use of some analgesics may influence study outcomes. For example, a study evaluating bone healing may need to avoid the use of NSAID as they are implicated in impairing the process of healing. Here we review the most commonly used analgesics in mice and rats including opioids, nonsteroidal antiinflammatory drugs (NSAID), local anesthetics, gabapentin, dissociative agents (ketamine), and acetaminophen, with particular application to surgical models of wound healing and orthopedics research.

Impact of analgesic choice on surgical orthopedic models.

Opioids. Opioids are the mainstay of pain control for human patients.³⁸ Opioids provide effective analgesia, however, the abundance of opioid receptors throughout the body means the potential for side effects is significant. These include sedation, respiratory depression, bradycardia, emesis, constipation, urinary retention, cognitive impairment, addiction, and tolerance in humans.^{9,13} Buprenorphine is most commonly used opioid for rodent models.^{29,55} Benefits of buprenorphine administration include its low toxicity, minimal effects on the immune system, and compared with other opioids, minimal respiratory depression.⁵⁵

The specific impact of opioids on rodent orthopedic models is still uncertain. Buprenorphine administration was shown to have a positive impact on tendon-to-bone healing in a gait analysis and tendon-bone healing ratio model.²³ Rats treated with buprenorphine (0.05 mg/kg SC, every 8 to 12 h, for 5 d) showed the greatest improvement as interpreted by gait analysis, limb loading, speed and force.²³ Others have confirmed the role of opioid receptors in the regulation of bone remodeling processes in rats. This was demonstrated by improved mechanical properties of bone following a 4 wk administration of either morphine (20 mg/kg/day SC) or buprenorphine hydrochloride (0.05 mg/kg/day SC) administration for 28 d in a model of osteoporosis.⁶⁴ Orthopedic models often result in chronic pain, which requires extended analgesia. In Sprague–Dawley rats, left femoral diaphysis fracture pain (where no analgesia was provided) was highest on day 1 postfracture; significantly decreased after 7 d; and returned to baseline (no pain) at 14 to 21 d.⁵⁰ Sustained-release buprenorphine (1.2 mg/kg, SC) provided analgesia for 2 to 3 d in a tibial defect model in male Sprague–Dawley rats, however, any secondary effect on the skeletal system was not specifically evaluated.⁴⁸ In humans, chronic opioid administration (including codeine, oxycodone, propoxyphene, or tramadol for greater than 3 mo) led to analgesic tolerance, a progressive lack of response to the drug.⁴² Opioid tolerance may also lead to hyperalgesia, a neural sensitization in which opioids can sometimes cause increased pain.^{75,114} In addition, chronic opioid administration may have adverse effects on bone healing. In a rat femoral fracture model, chronic morphine administration (5 mg/100 μ L/kg every 8 h for 8 wk) decreased fracture callus remodeling and resorption.²⁷ Taken together, the available data suggest that a short course (2 to 3 wk) of opioid analgesia (namely buprenorphine) will not negatively impact bone healing. Because of buprenorphine’s high-therapeutic index, analgesic effectiveness, and minimal negative effects on experimental outcomes in rodent orthopedic models, the authors recommend this analgesic as the primary analgesic choice for rodent orthopedic models.

Tramadol is an opioid agonist that functions by inhibiting serotonin and noradrenaline reuptake at the spinal cord. It may

be used as either an alternative to or adjunct to the aforementioned opioid analgesics. Both oral and injectable formulations are available.^{34,47} Tramadol (10 mg/kg, IP) and gabapentin (80 mg/kg, SC) administered every 8 to 12 h for 5 d was found to provide a good alternative to other analgesics postoperatively in a rat rotator cuff tendon-to-bone healing model, however the analgesic effect is not as good as with an opioid such as buprenorphine (0.05 mg/kg SC every 8 to 12 h for 5 d).²³

Little published information is available regarding the effect of tramadol on bone healing. Tramadol did not significantly inhibit human osteoblast activity *in vitro*.⁵¹ Furthermore, bone healing was not significantly impacted by low (5 mg/kg) or high (20 mg/kg) doses of tramadol subcutaneously daily for 6 wk in Sprague–Dawley rats.¹⁴²

Nonsteroidal antiinflammatory drugs (NSAID) and steroids. NSAID are valuable as a component of a multimodal analgesic regime. Physicians view NSAID as the safest, most effective way to treat postoperative pain.⁸⁵ Although NSAID are effective at reducing musculoskeletal pain, scientists may be hesitant to use this class of analgesics because of their potential to impact bone healing.^{32,110} NSAID reduce pain by blocking cyclooxygenase (COX) enzymes (COX1 or COX2), leading to inhibition of the production of PGE₂, an inflammation-moderating prostaglandin. Selective PGE₂ agonists have been shown to speed up bone healing.^{19,108} Multiple reviews have evaluated the effects of NSAID on bone healing without achieving a general consensus regarding its effects.¹¹⁰ For example, animal studies have indicated the following nonselective and selective COX inhibitors result in impaired bone healing: acetylsalicylic acid (ASA),³ ibuprofen,⁴ ketoprofen,⁸⁶ etodolac,⁴¹ meloxicam¹¹³ and diclofenac.⁷⁸ Others demonstrate no effect on bone healing after administration of: ibuprofen,⁹⁶ ketoprofen¹²⁹ or diclofenac.¹²⁸ A third group of studies showed no effect on bone healing with short-term use of either ketoprofen¹⁰⁰ or diclofenac.^{76,110} Further experimental studies (see Table 1) suggest that the effect of NSAID depends on the drugs themselves (that is dosages, dosing frequency, time of studies either early stages or later stages of bone healing); the specific animal models used: types and locations of bone fracture; the species, strains, ages, sexes, and numbers of animals used in each study; and the measurement methods and duration of the end-point of studies.^{10,32}

Nonselective COX inhibitors. Studies in mice suggest that nonselective COX inhibitors can negatively impact orthopedic healing (see Table 1). Indomethacin (2 mg/kg/day PO) and ibuprofen (30 mg/kg/day PO) were reported to inhibit fracture healing when administered either short- (less than 1 wk) or long-term (4 to 8 wk).¹⁶ Although ibuprofen (20 mg/kg PO administered preoperatively and every 8 to 12 h for 5 d after surgery) provided adequate early-stage postoperative analgesia, when compared to buprenorphine (0.05 mg/kg SC every 8 to 12 h for 5 d after surgery) and tramadol (10 mg/kg IP every 8 to 12 h for 5 d after surgery), it was found to impair bone healing in rats.²³ Ketorolac (4 mg/kg PO every 24 h for 21 d) was found to delay femoral fracture healing in male Sprague–Dawley rats more than parecoxib (1.5 mg/kg PO every 24 h for 21 d), a selective COX2 inhibitor.⁵³ Indomethacin (3 mg/kg SC for 7 wk) delayed spinal fusion in rats.³⁶ In a rat femur osteotomy model, short-term administration of indomethacin (200 mg/kg PO daily for 3 d) negatively impacted bone strength.⁶⁰ Because of the significant impact nonselective COX inhibitors had on bone healing, these drugs are not recommended for analgesia in orthopedic models.

Selective COX2 inhibitors. Long-term use of COX2 selective NSAID negatively impacts orthopedic models. In rats,

diclofenac (5 mg/kg PO daily for 10 d) reduced osteoblast proliferation and migration, delaying bone defect healing at the distal femur.⁷⁶ Carprofen (2.2 mg/kg BID for 4 mo) inhibited bone healing in a canine model of tibial osteotomy.¹⁰³ Rofecoxib administration reduced blood flow and impaired fracture healing in a mouse transverse middiaphyseal femoral fracture model (5 mg/kg PO for 32 d)⁹⁷ and rabbit fibula osteotomy model (12.5 mg PO daily for 28 d).¹⁰¹ In contrast, short-term administration of COX2 selective NSAID does not have a significant impact. Meloxicam (0.2 mg/kg/d IM for 10 d) impaired the fibular fracture healing process in Sprague–Dawley rats, yet the authors concluded there was not sufficient evidence of healing impairment to discourage use.⁶¹ In rats, celecoxib (2 mg/kg/day PO for 5 d) reduced femur fracture callus mechanical strength during early stages of bone healing, but there was no significant effect when used prior to fracture or 2 wk after.¹²³ Another study in rabbits indicated that Rofecoxib (12.5 mg PO daily for 2 wk) did not interfere with bone ingrowth of the proximal medial tibial metaphysis when given during the initial or final 2 wk of treatment.⁵⁴ Taken together, these data suggest that COX2 selective NSAID may be useful as part of a short-term (that is, less than 10 d) multimodal analgesic regimen for orthopedic models. In the short-term, the benefits of adding NSAID as part of the analgesic plan outweighs any potential delays in healing.

Steroids. Steroid administration during fracture healing delays the process as steroids can decrease osteoblastic activity, growth factor production and matrix synthesis.^{7,16,31} Long-term administration of prednisolone (0.15 mg/kg SC daily for 6 wk) inhibited bone healing in a rabbit ulnar osteotomy model.¹³⁵ Studies in rats⁷² (prednisolone pellets 1.82 to 2.56 mg/kg/day SC for 90 d) and mice¹³² (dexamethasone 2 to 4 mg/L in the drinking water for 12 wk) confirmed their susceptibility to glucocorticoid induced osteonecrosis. The effects of short-term steroid use are less well defined. In a rat femur osteotomy model, treatment with 3 consecutive injections of methylprednisolone (200 mg/kg IM daily for 3 d) had no significant effect on bone healing strength.⁶⁰ Because steroids have direct adverse effects on osteoblasts, osteoclasts, osteocytes and cartilage their use is not recommended for analgesia in orthopedic models.

Local anesthetics. Local anesthetics, (LA) can be used to provide analgesia to a specific area during intraoperative anesthesia and during the postoperative period. Local anesthetics diffuse across the plasma membrane and reversibly block voltage-gated sodium channels.¹²² They are the only analgesic drug class that completely disrupts the transmission of nociception to the central nervous system. Local anesthesia offers the benefit of ease of application, simplicity and few side effects.⁵⁷ The effect of lidocaine or bupivacaine (0.3 mL of either 1% lidocaine with epinephrine, 1% lidocaine without epinephrine or 0.25% bupivacaine) was evaluated when administered at the fracture site in a rat femoral fracture model, and there were no differences in callus composition, tensile strength or histologic appearance during early fracture repair.⁵⁹ Based upon the available information, local anesthetics are a good addition as part of a multimodal analgesic regimen.

Impact of analgesic choice for wound healing models.

Opioids. Opioid receptors and ligands are a part of the skin's neuroendocrine system and opioids are widely used to control wound pain.^{15,105} Many cells within the dermis are known to have opioid receptors. Keratinocytes, fibroblasts, neurons, and melanocytes are all known to express δ , μ and κ receptors. In early phase of wound healing, endogenous opioids are released

Table 1. Summary of cited studies evaluating the impact of analgesics used in rats or mice on orthopedic models.

Drugs	Dose, route, frequency	Strain/Species/Sex	Models	Effect on Model	Ref
Opioids					
Buprenorphine	0.05 mg/kg SC every 8-12 h for 5 d	Sprague–Dawley rats; male	Rotator cuff tendon-to-bone healing	No effect	Caro and colleagues ²³
Morphine or Buprenorphine	20 mg/kg/day SC 0.05mg/kg/day SC for 28 d	Wistar rats; female	Osteoporotic changes	Positive	Janas and colleagues ⁶⁴
Morphine	5mg/100µL/kg every 8h for 8 wk	Sprague–Dawley rats; unknown sex	Femur diaphyseal fracture model	Negative (decreased callus strength)	Chrastil and colleagues ²⁷
Tramadol	Tramadol low (5 mg/kg) or high (20 mg/kg) dose SC daily for 6 wk	Sprague–Dawley rats; female	Femur fracture model	No effect	Caro and colleagues ²³
Nonselective NSAID					
Indomethacin or Ibuprofen	2 mg/kg/day <i>PO</i> or 30 mg/kg/day <i>PO</i> for 1 or 8 wk	Mice; unknown sex	Femur fracture model	Negative	Boursinos and colleagues ¹⁶
Ibuprofen	20 mg/kg <i>PO</i> every 8 to 12 for 5 d	Sprague–Dawley rats; male	Rotator cuff tendon-to-bone healing	Negative	Caro and colleagues ²³
Ketorolac	4 mg/kg <i>PO</i> every 24 h for 21 d	Sprague–Dawley rats; male	Femur fracture model	Negative	Gerstenfeld and colleagues ⁵²
Selective NSAID					
Parecoxib	1.5 mg/kg <i>PO</i> every 24 h for 21 d	Sprague–Dawley rats; male	Femur fracture model	No effect	Gerstenfeld and colleagues ⁵²
Diclofenac	5 mg/kg <i>PO</i> daily for 10 d	Wistar rats; male	Femur defect model	Negative	Krischak and colleagues ⁷⁶
Rofecoxib	5 mg/kg <i>PO</i> for 32 d	Mice; unknown sex	Femur fracture model	Negative	Murnaghan and colleagues ⁹⁷
Celecoxib	2 mg/kg/day <i>PO</i> for 5 d	Sprague–Dawley rats; unknown sex	Femur fracture Model	No effect	Simon and O'Connor ¹²³
Meloxicam	0.2 mg/kg/d IM for 10 d	Sprague–Dawley rats; male	Fibular fracture model	Negative	Inal and colleagues ⁶¹
Steroids					
Prednisone	Pellets 1.82-2.56 mg/kg/day SC for 90 d	5 different inbred strains of rats; unknown sex	Glucocorticoid induced osteonecrosis	Negative	Kerachian and colleagues ⁷²
Dexamethasone	2-4 mg/L in the drinking water for 12 wk	BALB/cJ mice; unknown sex	Glucocorticoid induced osteonecrosis	Negative	Yang and colleagues ¹³⁸
Methyl-prednisolone	200 mg/kg IM daily for 3 d	Rats; unknown sex	Femur osteotomy	No effect	Hogevold and colleagues ⁶⁰
Local Anesthetics					
Lidocaine or Bupivacaine	0.3 mL of lidocaine or bupivacaine (1% lidocaine with epinephrine, 1% lidocaine without epinephrine or 0.25% bupivacaine)	Long–Evans rats; male	Femur fracture model	No effect	Henry and colleagues ⁵⁸

Notes: negative generally indicates impairment of healing and repair, while positive generally indicates promotion of healing and repair.

from inflammatory cells at the site of injury and have been found to promote ischemic wound healing.¹³⁴

Apart from their analgesic properties, opioid agonists modulate angiogenesis, a critical process for wound healing, while opioid antagonists (for example, naltrexone) have antiangiogenic effects.¹⁰⁵ A study in mice indicated that the μ -opioid receptor plays a critical role in the proliferation phase of wound healing and in granulation tissue formation.¹³⁴ In rats, topical administration BID for 7 d of an opioid preparation (fentanyl,

5 μ g/g, hydromorphone, 0.2 mg/g or morphine, 1.5 mg/g) blended with a hydrocream base hastened midline wound closure by increasing granulation tissue and collagen formation, epidermal and dermal organization, and angiogenesis.¹⁰⁹ In an *in vitro* wound healing model, topical opioid administration via addition to the cell media for 48 h (including morphine, hydromorphone, fentanyl, and buprenorphine) accelerated wound closure.¹³⁷ However, long-term high-doses of morphine (20 mg/kg IP daily for 14 d) delayed vascularization and closure

of excisional skin injury in mice.⁸⁰ Thus, because of the potential delay of wound healing with sustained use of buprenorphine (greater than 10 d), the authors recommend only short-term use of buprenorphine for acute surgical wound models.

Tramadol used as a local infiltration at the surgical site (1 mL of 2.5% tramadol) did not have adverse effects on wound healing in Wistar Albino rats.¹⁰⁶ Infiltration with tramadol (3 mL of 5% tramadol) reduced postoperative pain and did not affect wound repair.⁵⁷ Consequently, tramadol can be used for wound infiltration anesthesia without impacting the wound healing process.

NSAID and steroids. Few studies have evaluated the effect of NSAID on wound healing. Proper wound healing requires hemostasis, inflammation, proliferation, and remodeling.^{56,99} NSAID have antiproliferative effects on vasculature and skin and can increase scarring and delay wound healing due to suppression of PGE₂.⁵⁶ A study in rats indicated that flunixin meglumine (1.1 mg/kg IM every 12 h for 14 d), reduced the inflammatory stage, but not the proliferative stage (when fibroplasia is a factor in eventual wound strength). Histologically, there were no differences in wound healing and strength between flunixin and nonflunixin treated groups.³⁸ Results are conflicting regarding the effects of COX2 selective NSAID on wound healing. A study in rats found that diclofenac (5 mg/kg PO twice daily for 10 d), reduced the number of fibroblasts and weakened epithelialization, although wound healing was not affected based upon clinical parameters.⁷⁶ Celecoxib administered in the diet (celecoxib 1500 ppm added to AIN-76A diet for 1 wk), was shown to delay wound healing in mice, but improve healing of pressure ulcers (5 mg/kg daily in water for 2 wk).^{45,117} Overall, studies of the direct effects of NSAID on wound healing are inconclusive (see Table 2); however, because of the benefits of analgesia we recommend short-term (less than 1 wk) use of NSAID for analgesia in wound healing models.

Steroids. Steroids are recognized to inhibit wound healing through antiinflammatory effects and suppression of cellular responses, including fibroblast proliferation and collagen synthesis. Steroids also suppress genes responsible for interleukin signaling, keratinocyte proliferation, and cytoskeleton remodeling, and inhibiting the immune system, thus increasing the risk of infection.¹²⁶ Methylprednisolone (single dose, 40 mg/kg IM), a long-acting synthetic steroid, was shown to suppress wound healing in male CD1 mice⁴⁵ and male Sprague–Dawley rats (single dose of methylprednisolone acetate, 6 mg SC).¹³⁶ Other work in various species has similarly demonstrated up to 30% reduction in wound tensile strength with corticosteroid dosages of 15 to 40 mg/kg/day.¹³³ Topical steroids also inhibit acute wound healing. For example, weak-strength ((hydrocortisone cream 1%) and medium-strength (fluocinolone acetonide ointment 0.025%)) steroid preparations were reported to inhibit acute healing in hamsters.⁸⁴ Taken together, these data suggest that systemic and topical steroid use should be avoided in wound healing models (see Table 2).

Local anesthetics. Infiltration of a local anesthetic agent into the wound site can be an effective way to reduce postoperative pain.¹¹⁵ LA affects the first 2 stages of wound healing, the inflammatory and proliferation stages.¹⁷ LA offer antiinflammatory effects by reducing eicosanoid, prostaglandins, thromboxanes, leukotrienes, histamine, inflammatory cytokines and the scavenging of oxygen free radicals.²⁴ LA also suppress synthesis of mucopolysaccharides; reduce collagenization; and reduce the quantity of mast cells at the wound site.^{94,116}

The data on the effects of local anesthesia on wound healing and breaking strength are conflicting (see Table 2).^{63,132} Some

studies indicate that lidocaine and bupivacaine inhibit collagen synthesis, cause cytotoxic effects, delay wound healing, and reduce wound breaking strength and collagen production.^{40,44,57} A single infiltration dose of lidocaine did not significantly affect wound breaking strength in rabbits (0.5% lidocaine SC)¹³¹ or rats (2% lidocaine, 2.5 mL injected in 4 different locations, SC).³⁷ However, another study in Sprague–Dawley rats reported that lidocaine infiltration (single infiltration, 2 mL of 0.5% to 2% lidocaine) impaired wound breaking strength at 5 to 7 d in a dose-dependent manner that was potentiated by epinephrine.⁹⁴ In that study, tensile strength was also impaired by injection of water, suggesting that some impaired healing was due to the physical damage resulting from injection of fluid into the skin and subcutaneous tissue.⁹⁴ Another study demonstrated a single 3 mL subcutaneous infiltration of either lidocaine or prilocaine had no effect on wound healing in Sprague–Dawley rats, although a similar dose of either bupivacaine or levobupivacaine negatively affected wound tensile strength at 21 d.⁷³ These results suggest that bupivacaine and levobupivacaine negatively affect late-stage wound healing, and lidocaine or prilocaine have no effect on late-stage wound healing. These studies indicate that the effect of the LA on wound healing is dependent on the concentration, volume, duration and anesthetic classification (see Table 2).

EMLA cream, an emulsion of lidocaine and prilocaine, is a commonly used topical anesthetic. Results from a study in guinea pigs indicated that application of 0.25 mL of EMLA cream resulted in an increased inflammatory response, and was associated with susceptibility to wound infection.¹¹¹ A more recent study in mice indicated that twice-daily topical EMLA application had a beneficial effect on experimental wound healing and resulted in increased tensile strength of the wound tissues.⁴²

Prolonged use of LA (for example via a wound catheter, a simple fenestrated tube placed at a wound for the purpose of continuous or intermittent administration of local anesthetics) or high LA concentrations should be used cautiously. The majority of studies regarding the effect of LA on wound healing were done at early stages of wound healing. It is unknown if there are lasting effects of LA on late-stage wound healing (that is, after remodeling). Liposomal bupivacaine is a prolonged-release formulation of bupivacaine HCl indicated for single-dose infiltration into the surgical site to produce postsurgical analgesia.¹ Our group did not see any significant impact on wound healing when used at 6 mg/kg SC in Sprague–Dawley rats.⁶⁹ In human patients, liposomal bupivacaine administered locally at the surgical wound site resulted in no clinically evident impact on wound or bone healing.¹¹ Due to the benefits of LA as a form of analgesia with minimal adverse effects, we recommend short-term (provided before or at the time of wound treatment) LA as acceptable in wound healing models.

Other analgesics for orthopedic and wound healing models. **Gabapentin.** Gabapentin (80 mg/kg IP) has been used as an adjunctive analgesic to tramadol (10 mg/kg IP) in rodents.⁹⁰ Gabapentin (1200 mg/day, oral gavage twice daily) negatively affected bone fracture healing in a model using female Wistar Albino rats.¹²⁵ However, gabapentin (20 mg/kg oral gavage 1 d before surgery and once and day for 9 d postsurgery) minimally affected wound healing in male Wistar Albino rats.¹¹⁸ These data support the use of gabapentin in wound healing but not orthopedic surgical models.¹¹⁸

Ketamine. Ketamine (low dose) can be used as a preventative analgesic against hypersensitivity because of its action as a NMDA antagonist.⁴⁷ Pretreatment with ketamine (10 mg/kg SC) was reported to attenuate morphine-induced hyperalgesia

Table 2. Summary of cited studies evaluating the impact of analgesics used in rats or mice on wound healing models.

Drugs	Dose, route, frequency	Strain/Species/Sex	Models	Effect on Model	Ref
Opioids					
Fentanyl,	Fentanyl (5 µg/g) twice daily	Fischer 344 rats; unknown sex	Wound healing	Positive	Poonwala and colleagues ¹⁰⁹
Hydromorphone, or Morphine	(0.2 mg/g) twice daily or 1.5 mg/g twice daily				
Morphine	20 mg/kg IP daily for 14 d	C57BL/6J mice; unknown sex	Excisional skin injury	Negative	Lam and colleagues ⁸⁰
Tramadol	1 mL of 2.5% tramadol as local infiltration	Wistar albino rats; unknown sex	Wound healing	No effect	Ozkan and colleagues ¹⁰⁶
Nonselective NSAID					
Flunixin meglumine	1.1 mg/kg IM every 12 h for 14 d	Sprague–Dawley rats; male	Wound healing	No effect	Donner and colleagues ³⁸
Diclofenac	5 mg/kg PO twice daily for 10 d	Wistar rats; male	Wound healing	No effect	Krischak and colleagues ⁷⁷
Selective NSAID					
Celecoxib	1500 ppm added to AIN-76A diet for 1 wk	C57BL6/J mice; unknown sex	Wound healing	Negative	Fairweather and colleagues ⁴³
Celecoxib	5 mg/kg daily in water for 2 wk	Swiss mice; male	Pressure ulcers	Positive	Romana-Souza and colleagues ¹¹⁷
Steroids					
Methyl-prednisolone	40 mg/kg IM once	CD1 mice; male	Wound healing	Negative	Fesser and colleagues ⁴⁵
Methyl-prednisolone	6 mg SC once	Sprague–Dawley rats; male	Wound healing	Negative	Wicke and colleagues ¹³⁶
Local Anesthetics					
Lidocaine	2% SC	F344 rats; unknown sex	Wound healing	No effect	Dogan and colleagues ³⁷
Lidocaine	2 mL of 0.5% to 2%	Rats; unknown sex	Wound healing	Negative	Morris and Tracy ⁹⁴
Lidocaine or Prilocaine	lidocaine 7 mg/kg local infiltration	Sprague–Dawley rats; unknown sex	Wound healing	No effect	Kesici and colleagues ⁷³
Bupivacaine or Levobupivacaine	2 mg/kg local infiltration or 2.5 mg/kg local infiltration	Sprague–Dawley rats; unknown sex	Wound healing	Negative	Kesici and colleagues ⁷³

Notes: negative generally indicates impairment of healing and repair, while positive generally indicates promotion of healing and repair.

in mouse bone fracture models.⁹³ When ketamine is used as a part of the rodent anesthesia plan, the subsequent surgical pain is reduced.⁴⁷ However, we were unable to find any published studies evaluating the impact of ketamine on wound healing.

Acetaminophen (Paracetamol/Tylenol). Acetaminophen is used as an oral analgesic in dogs and small mammals.^{35,47,87} Acetaminophen did not negatively affect bone fracture healing in Sprague–Dawley rats (6 to 300 mg/kg PO for 10 d)^{13,48} or strength of healing of the intestinal or abdominal fascia (anastomotic and abdominal wall healing model) in male Wistar rats (50 to 200 mg/kg/d PO for 3 to 7 d).¹³⁰ Others have described a lack of any effect on wound healing strength in a rat patellar tendon resection model when animals were administered acetaminophen (60 mg/kg for 7 d in the diet).⁴⁶ Acetaminophen can be used as an adjunct analgesic for orthopedic or wound healing surgical models.

Multimodal analgesia. Despite the known benefits of multimodal analgesia, few reports evaluate its use.^{21,127} To our

knowledge, data are not available that specifically evaluate the effects of a multimodal analgesic regime on either bone or wound healing in rodents. One study used a combination of tramadol (10 mg/kg IP, q 8 to 12 h for 5 d) and gabapentin in a male Sprague–Dawley rat tendon-to-bone healing model.²² The study found no significant differences in healing, and the multimodal regimen was less efficacious in controlling postsurgical pain than was buprenorphine (0.05 mg/kg SC q 8 to 12 h for 5 d) or ibuprofen (10 mg/kg IP). Using other multimodal analgesic regimes (that is buprenorphine in combination with NSAID and/or local anesthetics) will increase the safety and efficacy of analgesia.⁹⁸ Given these data, we propose that best practice is to provide multimodal analgesia during rodent surgery.

Conclusion

Refinement of the analgesic plan. The majority of orthopedic and wound healing models cause moderate to severe pain. A

Table 3. The below table summarizes the authors' recommendations regarding the impact of analgesic choice on the surgical model

	Opioids	NSAID	Steroids	LA	Other
Orthopedic models—bone (fracture)	Recommend (buprenorphine, tramadol or local infiltration of tramadol)	Use caution (may consider short-term selective COX2 inhibitors)	Avoid	Recommended	Consider adjunctive analgesia with tramadol, ketamine, acetaminophen
Wound healing	Recommend (short-term buprenorphine; topical use of opioids)	Use caution (may consider short-term selective COX2 inhibitors)	Avoid	Use caution	Consider adjunctive analgesia tramadol, gabapentin, ketamine, acetaminophen

well-designed analgesic plan must be implemented by working closely with veterinarians familiar with the species being used. When possible, preemptive, multimodal analgesic techniques should be used to reduce the need for subsequent analgesic doses, speed recovery and wound healing, and to prevent chronic pain.

The analgesic choice depends on many factors: the dose, duration interval, route of administration, surgical model, species, strain, sex, and health. Other factors to consider are the skill of the surgeon and the amount of tissue trauma inherent in the model. Inexperienced surgeons may cause more tissue trauma than more skillful practitioners by applying excessive pressure or causing ischemia during hemostasis, which may require higher doses of pain relief or extended analgesic coverage. Training in proper surgical technique and anatomic knowledge relevant to the model studied is thus necessary to uphold the 3Rs principle of refinement.⁹⁸ IACUC must critically evaluate surgeon skill and possibly require additional training such as surgeon shadowing, practicing survival surgeries on mice that are euthanized immediately after procedures, and/or surgical training on cadavers/inanimate models. Surgeons must consider how to reduce surgical time by working with a surgical technician or an anesthetist. The IACUC and veterinary staff must follow-up with researchers and work closely with them during surgical model development to ensure technical proficiency and animal welfare. Furthermore, if researchers will be responsible for postoperative care, they must be properly trained to detect pain/distress, evaluate animals for early euthanasia criteria and humane endpoints. Researchers must also understand how to contact the veterinary staff to request additional analgesia and supportive care as defined in the approved IACUC protocol. Regardless of the analgesia provided, non-pharmacologic treatment must always be considered as an adjunctive measure for analgesia in the postoperative period. Factors such as additional enrichment, social housing, soft bedding, nesting materials, and accessible and palatable food and water supplements may improve animal welfare, but should not replace chemical analgesia.

Concerns about the effects of analgesic use on experimental outcomes in both orthopedic and wound healing models may influence and possibly limit drug choice. Although full μ -opioids or NSAID can have negative effects on the orthopedic and wound healing models, limited information is available regarding other types of opioids (that is buprenorphine SR, butorphanol, or tramadol). Further evidence-based and risk-benefit ratio studies (especially with NSAID) are needed. In the authors' opinion, if NSAID are used in orthopedic models, short-term use of selective COX2 inhibitors and/or local anesthetics would be appropriate.¹¹⁰ If infiltration of local anesthetics at the surgical site is contraindicated, researchers might consider using a regional nerve block using a LA (blocking the nerve proximal to surgical sites using a nerve locator). Prudent, short-term use of opioids or NSAID for pain associated with

wound healing is appropriate, unless a clear scientific justification exists (see Table 3). No single universal analgesic plan can be applied uniformly to complicated surgical models, such as those used in orthopedic and wound healing research. Further studies designed to examine the efficacy and experimental effects of various analgesic regimens, in a variety of species and specific surgical models are needed for further refinement. In all cases, we stress the importance for veterinarians and researchers to work together closely to devise an optimal analgesic plan for the specific surgical model.

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