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Orthopedic surgery and bone fracture pain are both significantly attenuated by sustained blockade of nerve growth factor

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Abstract

The number of patients suffering from postoperative pain due to orthopedic surgery and bone fracture is projected to dramatically increase because the human life span, weight, and involvement in high-activity sports continue to rise worldwide. Joint replacement or bone fracture frequently results in skeletal pain that needs to be adequately controlled for the patient to fully participate in needed physical rehabilitation. Currently, the 2 major therapies used to control skeletal pain are nonsteroidal anti-inflammatory drugs and opiates, both of which have significant unwanted side effects. To assess the efficacy of novel therapies, mouse models of orthopedic and fracture pain were developed and evaluated here. These models, orthopedic surgery pain and bone fracture pain, resulted in skeletal pain-related behaviors that lasted 3 weeks and 8 to 10 weeks, respectively. These skeletal pain behaviors included spontaneous and palpation-induced nocifensive behaviors, dynamic weight bearing, limb use, and voluntary mechanical loading of the injured hind limb. Administration of anti-nerve growth factor before orthopedic surgery or after bone fracture attenuated skeletal pain behaviors by 40% to 70% depending on the end point being assessed. These data suggest that nerve growth factor is involved in driving pain due to orthopedic surgery or bone fracture. These animal models may be useful in developing an understanding of the mechanisms that drive postoperative orthopedic and bone fracture pain and the development of novel therapies to treat these skeletal pains.

Keywords

NGF; Orthopedic surgery; Bone fracture; Pain

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Appendices. Supplemental Digital Content

The appendices are available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A18> (fig.1), <http://links.lww.com/PAIN/A19> (fig.2), <http://links.lww.com/PAIN/A20> (Table 1), <http://links.lww.com/PAIN/A21> (video 1), and <http://links.lww.com/PAIN/A22> (video 2).

1. Introduction

As the life span of the world's population continues to rise, experts predict that there will be a dramatic increase in the number of orthopedic surgeries required for knee or hip replacements and surgeries to stabilize, align, and repair bone fractures.^{19,42,56} For example, in 2010, there were 719,000 knee replacement surgeries performed in the United States, and this number is projected to increase to 3.5 million by 2030.⁴² Currently, it is estimated that there are 1.6 million hip fractures worldwide, and this number is projected to reach 6.3 million by 2050.¹⁷ What orthopedic surgery and bone fracture share in common is that adequate control of pain is usually required for the patient to fully participate in the physical rehabilitation, which requires mechanical loading of the operated bone or joint.^{2,11} If this pain is not adequately controlled, especially in the severely injured and elderly, it can interfere with effective rehabilitation and skeletal healing, resulting in significant loss of bone and muscle mass, and ultimately leading to the development of chronic skeletal pain.^{22,41}

Currently, the most common classes of pharmacological agents used to treat post-orthopedic surgery pain are nonsteroidal anti-inflammatory drugs (NSAIDs) and/or opiates. Both of these classes of drugs have significant unwanted side effects,^{55,57} and some of these drugs may directly or indirectly inhibit bone healing and/or functional recovery.⁵⁸ Nonsteroidal anti-inflammatory drugs (including ibuprofen, COX-2 inhibitors, naproxen, and diclofenac) have been shown to inhibit bone healing in rodent fracture models,^{1,25,57} and if used over an extended period of time in humans, can also have unwanted renal, hepatic, and gastrointestinal side effects.⁵⁵ In light of these issues with NSAIDs, it has now become more common for opiates to be used to control moderate-to-severe post-orthopedic surgery skeletal pain.⁴⁷ However, recent data have suggested that although opiates can be useful in controlling nonmalignant skeletal pain for 2 to 3 months after orthopedic surgery, long-term use (>2-3 months) is associated with lack of return to functional status, inability to return to work, as well as potential development of dependence, constipation, and respiratory depression.^{39,61,69} In older individuals, which is the population that has the greatest number of orthopedic procedures, opiates are also more likely to induce dizziness, vertigo, and cognitive clouding, all of which can result in further falls producing new fractures or reinjury to the operated bone or joint.¹⁴

In the present report, we develop and evaluate 2 preclinical models that measure several end points that are commonly used in human patients who have bone fractures or have undergone elective joint replacement. Using these models, we then examined whether administration of an antibody that sequesters nerve growth factor (anti-NGF) can provide relief from orthopedic surgery pain or bone fracture pain. The data presented here suggest that anti-NGF is efficacious in reducing orthopedic surgery pain and bone fracture pain using behavioral end points that are similar to those used to assess skeletal pain in humans.

2. Methods

2.1. Animals

Experiments were initiated with 92 adult male C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME), approximately 2 to 3 months old, weighing 25 to 30 g. The mice were housed in accordance with the National Institutes of Health guidelines under specific pathogen-free conditions in autoclaved cages maintained at 22°C with a 12-hour alternating light/dark cycle and had access to food and water ad libitum. All procedures adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Institutional Animal Care and Use Committee at the University of Arizona (Tucson, AZ).

2.2. Surgical and fracture procedures

2.2.1. Femoral pin placement—After induction of deep anesthesia with ketamine/xylazine (0.01 mL/g, 100 mg/kg ketamine, and 10 mg/kg xylazine, subcutaneously [s.c.]), mice were prepared for surgery by shaving and swabbing the lateral hip and leg area with Betadine followed by 70% ethanol (repeated twice). Mice were then placed on their side on heating pads (Braintree Scientific, Braintree, MA), and a 1-cm incision was made in the skin of the upper hind limb (parallel to the femur) to expose the muscle. The skin was separated from the underlying muscle, and an incision was made between the rectus femoris and vastus medialis muscles. The rectus femoris muscle and patella were displaced medially, exposing the femoral condyles. Using a dremel fitted with a 0.5-mm bit, a hole was drilled between the condyles in the middle of the patellar groove (aligned parallel with the femur). A C313I injection cannula (Plastics One, Inc, Roanoke, VA) was inserted into the intramedullary space to make a cavity in the bone marrow. A precut 0.011 inch diameter stainless steel wire (Small Parts, Inc, Logansport, IN) was inserted for fracture stabilization. An x-ray was taken to confirm pin placement. The insertion site was sealed with bone cement, and the patella was gently returned to its original position using thumb and forefinger. The muscles were secured using a horizontal mattress suture technique with absorbable sutures. The injection site was well irrigated with sterile saline, and wound closure was achieved with two 7-mm auto wound clips (Becton Dickinson, Sparks, MD). Animals recovered from anesthesia on heating pads and received injections of antibiotic (10 mg/kg Amikacin, intramuscularly) and sterile saline (1 mL, s.c.). After surgery, mice were housed either individually or 3 per cage. Wound clips were removed on day 7 after surgery.

2.2.2. Fracture—A closed mid-diaphyseal fracture of the femur was produced 21 days after pin placement in mice under ketamine/xylazine anesthesia using a 3-point bending device (BbC Specialty Automotive Center, Linden, NJ) based on the fracture apparatus described by Bonnarens and Einhorn.⁹ The anesthetized mice were placed in a supine position with the femur (medial side up) directly over the support anvil of the bending device. The blunt guillotine blade was gently lowered onto the hind limb equidistant between the knee and the hip joints. A 168-g weight was dropped onto the guillotine from a height of 19.8 cm, creating the closed fracture. Immediately after fracture, mice were radiographed to ensure the localization of fracture at the mid-diaphysis of the femur (± 1.5 mm). There were 36 mice that met at least 1 of the following exclusion criteria and were

removed from the study: (1) fractures located too far from the mid-diaphyseal region of the femur, (2) dislodged pin, (3) invisible fracture after impact, and (4) fragmentation of the bone. The remaining 56 mice continued through behavioral testing. The diagram of 3-point bending device is given in the appendices, available online as Supplemental Digital Content.

2.3. Behavioral measures of bone fracture pain

Mice were assessed for baseline (presurgery), orthopedic surgery (pin placement), and fracture-induced pain behaviors. For orthopedic surgery, animals were assessed on day 7 (baseline) and on days 1, 3, 7, 10, 14, 21, and 28 after pin placement. After fracture, animals were assessed at weeks 0, 1, 2, 3, 4, 5, 7, 9, and 11. Behavioral testing was performed on the same days as radiological assessment to enable comparison between pain behavior and bone fracture healing. Each method of behavioral measure assessment was performed by the same experimenter.

2.3.1. Spontaneous nocifensive behavior—Mice were placed in a large Plexiglas box raised 20 inches above the surface of the bench. The box is divided into 20 chambers ($11.5 \times 6.8 \times 7.5$ cm) with wire grid floors. The mice were allowed to acclimate for 30 minutes (until cage exploration and major grooming activities cease), and then their movements were videotaped from below using Sony Handycam DCR-SR68 cameras. Time spent in “nocifensive” behavior was assessed over a 5-minute observation period (between minutes 15 and 20 of the filmed behavior). Nocifensive behavior was defined as (1) full guarding (lifting the affected limb and holding it against its body), (2) reduced weight bearing (affected limb is held in such a way that the foot, or the side of the foot, is merely resting on the floor), (3) tending to the affected limb (abnormal grooming behavior directed solely to affected limb, specifically licking lower limb and foot), (4) flinching the affected limb, and (5) sporadic hopping (intermittent jumps without using affected limb). A video depicting these behaviors can be found in the appendices.

2.3.2. Dynamic weight bearing—The percentage of weight borne by each limb of a freely moving animal was measured using a floor-instrumented dynamic weight-bearing system ([DWB]; Bioseb EB Instruments, Pinellas Park, FL). The mouse was placed in a small Plexiglas cage ($11 \times 11 \times 22$ cm), and a camera was placed on top of the enclosure. The animal was allowed to move freely within the apparatus for 5 minutes, while the pressure data and live recording were transmitted to a laptop computer through a USB interface. The data were stored on the computer for subsequent analysis. After the completion of each test, the mouse was removed and the test chamber was cleaned with alcohol wipes to reduce the potential for stress-induced analgesia as a result of any stress odor from the previous animal. For data analysis, the raw pressure data were automatically synchronized with images from the video camera, and the averaged values were encrypted and rerecorded on a computer. Using the BioSeb software v1.3, the operator then manually “validated” each test period, ensuring each print corresponded to the appropriate paw using the synchronized video feed as a reference. A zone was considered valid when the following parameters were detected: 4 g on 1 captor with a minimum of 2 adjacent captors recording 1 g. For each time segment where the weight distribution was stable for more than 0.5 seconds, zones that met the minimal criteria were then validated and assigned as either right

or left hind paw or front paw by the experimenter according to the video and the scaled map of activated captors. Data are presented as percent weight borne by ipsilateral hind limb of total weight borne by both hind limbs.

2.3.3. Rearing—The number of times an animal reared (simultaneously lifted both front paws up from the floor) over a 5-minute period was recorded as a measure of voluntary activity. Rearing was assessed during the time when the animal was freely moving in the DWB apparatus described in section 2.3.2.

2.3.4. Palpation-induced nocifensive pain behavior—After testing for fracture-induced spontaneous pain on weeks 3, 5, 7, 9, and 11, mice were gently palpated and reassessed for bone fracture pain to assess bone fracture healing, including nocifensive behaviors, dynamic weight bearing, and rearing as described. Using thumb and forefinger, the investigator gently and repeatedly pressed the thigh of the fractured hind limb (1 palpation per second) for 2 minutes. Palpation-induced pain measurements correlate with pain that is clinically relevant to a patient undergoing orthopedic treatment.

2.4. Radiography

High-resolution x-ray images of the mediolateral plane of the ipsilateral (fracture site) femur were obtained after gentle anesthesia of mice with ketamine/xylazine (0.005 mL/g, 50 mg/kg ketamine, and 5 mg/kg xylazine, s.c.) (1) several days before pin placement surgery (baseline) and weekly after pin placement surgery to assess recovery from pin placement (animals were excluded from the study if a patella displacement was identified through radiography to have occurred), and (2) before and after fracture procedure, and weekly following fracture to assess fracture healing. X-rays were taken using a Faxitron MX-20 digital cabinet x-ray system (Faxitron/Bioptics, Wheeling, IL).

2.5. Treatment with anti-nerve growth factor

The anti-NGF sequestering antibody (mAb 911 provided by Dr David Shelton; Rinat/Pfizer, San Francisco, CA) is effective in blocking the binding of NGF to both tropomyosin receptor kinase A (TrkA) and p75 NTR receptors and inhibiting TrkA autophosphorylation.⁵⁴ The anti-NGF antibody possesses a plasma half-life of approximately 5 to 6 days in the mouse, and it does not appreciably cross the blood-brain barrier.⁶⁵ The dose used in this study (10 mg/kg, intraperitoneally [i.p.]) has been shown to be efficacious at attenuating fracture pain in mice at a dose of 10 mg/kg, i.p., when given every 5 days.³⁷ Therapy was initiated either before orthopedic surgery was performed or 1 day after fracture. Mice were divided into 4 groups: orthopedic surgery + anti-NGF, orthopedic surgery + vehicle, fracture + anti-NGF, and fracture + vehicle. The general health of the mice was closely monitored, with general appearance and body weight used as general health indicators throughout the experiments.

2.6. Statistical analysis

A 1-way analysis of variance or *t* test was used to compare behavioral results and bone scores between the experimental groups. Significance level was set at $P < 0.05$. In all cases,

the investigator responsible for behavioral testing, plotting, measuring, and counting was blinded to the experimental situation of each animal.

3. Results

3.1. Fracture protocol overview

We describe a series of critical end points and inclusion criteria applied in our animal model of orthopedic surgery and bone fracture pain to obtain clinical relevance. **Figure 1** depicts high-resolution x-ray images of a representative C3H femur at naive (baseline), pin placement, fracture, and healing evolved over time. The 3-point fracture protocol resulted in reproducible transverse or slightly oblique mid-diaphyseal femoral fractures (white arrows). Mineralized callus formation surrounding the fracture line can be visualized by radiographs on day 10 after fracture. On day 14 after fracture, the mineralized callus is most prominent in size (not shown) and undergoes a time-dependent reduction in size, as shown in subsequent radiographs. Studies show that cortical union becomes apparent as of week 7 after fracture.⁶⁶

3.2. Nocifensive behavioral assessment of orthopedic and bone fracture pain

In the orthopedic surgery group, spontaneous nocifensive behavior was assessed over a 5-minute period before pin placement surgery and on days 1, 3, 7, 10, 14, and 21 after surgery. Nocifensive behavior was assessed for the fracture mice on weeks 0, 1, 2, 3, 4, and 5 after fracture (**Fig. 2A**). After the pin placement procedure, mice displayed significantly more spontaneous nocifensive behaviors when compared with baseline (t test, $P < 0.05$). This orthopedic surgery pain is maintained for 3 weeks after the pin placement. Mice returned to presurgical baseline behaviors 4 weeks after orthopedic surgery (week 0 on timeline). On bone fracture production, mice displayed significantly greater spontaneous nocifensive behaviors over the course of a month and approached baseline 5 weeks after fracture. Graphs of results for limb use and dynamic weight bearing after fracture appear in Supplemental Materials (Fig. 2).

3.3. Anti-nerve growth factor reduces both orthopedic and fracture pain

Chronic treatment with anti-NGF (10 mg/kg, i.p.) administered before orthopedic surgery and on days 1, 6, and 11 after surgery reduced the orthopedic surgery-induced skeletal pain ranging between 31% and 70% when compared with vehicle-treated mice (**Fig. 2B**). Spontaneous pain was significantly reduced on days 1 and 11 after orthopedic surgery. Chronic treatment with anti-NGF administered on days 1, 6, 11, and 16 after closed femur fracture reduced fracture-induced skeletal pain between 39% and 71% and was significant on days 4 and 21 after fracture (**Fig. 2C**).

3.4. Prepalpation and postpalpation pain-related behavior

Our data show that while spontaneous nocifensive behaviors return to baseline within 7 weeks after fracture, the time spent in nocifensive behaviors is increased after palpation of the mouse femur (**Fig. 3A**). This palpation-induced increase in pain is maintained until week 11, after which palpation ceased to elicit nocifensive behavioral responses. There is a significant reduction in rears after palpation on days 3, 7, and 9 (**Fig. 3B**), but palpation

caused no effect at week 11. Dynamic weight bearing showed no significant difference after palpation during these time points (data not shown). Exploratory studies suggested that performing palpation before significant callus formation (before day 14) resulted in a higher proportion of animals that showed displacement of the fracture and nonunion or re-fracture of the affected bone and therefore were not performed in this study. These measures of functional status are not only important in the clinic but also for more accurate assessment of novel treatment efficacy in preclinical studies.

3.5. Critical alterations to surgical and experimental methods improve outcome

3.5.1. Exclusion of mice with patella displacement—As described above, patella manipulation, drilling, and the placement of a stainless steel pin to stabilize the bone before fracture caused a significant but short-lived orthopedic surgical pain. When performing the pin placement surgery, care must be taken to avoid patellar displacement (**Figs. 4A-D**) after the animals recover from surgery. Animals with patellar displacement (black squares, **Fig. 4E**) had significantly higher pain responses on days 7, 11, and 14 after pin placement surgery when compared with animals without patellar displacement (white triangles, **Fig. 4E**). After we observed that patellar displacement can skew behavioral testing results, once the patella was returned to its original position, we used a modified mattress suture technique to secure the patella in place. Other significant improvements to the surgical techniques developed included making the 1-cm skin incision on the hip instead of over the knee and using a mock injector cannula to make a canal in the medullary space before pin insertion. Combined, these improvements significantly reduced the instances of patellar displacement while improving recovery (data not shown).

3.6. Avoidance of nonsimple fracture development by singly housing mice

After fracture production, animals that were group housed experienced more instances of simple fractures that became nonsimple and more complex (as shown in radiographs taken on day 7) (**Fig. 5A**), when compared with animals that were housed singly (simple fractures on day 7, **Fig. 5B**). By day 7 after surgery, 73% of animals housed 3 animals per cage that originally had a simple fracture on day 0 had a nonsimple, displaced, or complex fracture (black bar, **Fig. 5C**) compared with less than 10% of animals housed individually that had simple fractures become nonsimple, displaced, or complex fracture by day 7 (gray bars, **Fig. 5C**). Note that the singly housed animals were minimally handled for the first 7 days after surgery.

3.7. Preclinical and clinical measurements

Table 1 translates the measures assessed in our preclinical mouse model of orthopedic surgery and femoral fracture, such as bone healing, pain, and functional status, to more clinically relevant measures routinely obtained by physical examinations in the clinic with human patients having undergone orthopedic surgery or received bone fracture injuries.

4. Discussion

In this study, a mouse model of orthopedic surgery and a model of bone fracture pain were further developed and evaluated. These 2 models of skeletal pain are in large part based on

the closed femoral fracture model that was originally developed to study fracture healing in the rat⁹ and mouse⁴⁸ and has been extensively used by bone researchers to study mechanisms that drive bone fracture healing.^{15,24,26,33,34,40,49} Another model that has been used to study fracture pain is one where the tibia is fractured, and then the fractured limb is wrapped in casting tape so that the hip, knee, and ankle are immobilized.²⁸ This model has also been used extensively to study the mechanisms that drive complex regional pain syndrome,^{4,23,29} where bone fracture is 1 of the common precipitating injuries that drives this very difficult to control pain state.^{5,30,52}

The major reasons the closed femoral fracture model was used in developing an orthopedic surgery and bone fracture pain model were that this model allowed us to monitor skeletal pain behaviors from the time of the initial surgery or fracture to the time when full skeletal healing has occurred, as well as measuring the effect that skeletal analgesics may have on bone healing. Additionally, many of the preclinical end points used in these pain models are similar to those used in the clinic to assess bone healing after joint replacement and bone fracture healing.^{18,53} For example, orthopedists frequently manually palpate the affected area of a patient's limb to more accurately determine the bone healing progress.^{20,32,35} We show here that although spontaneous nocifensive behaviors return to baseline by 7 weeks after fracture, the pain behaviors remain heightened after palpation of the fractured area for up to 10 weeks, well after cortical union of the mineralized bone has occurred. Interestingly, human studies have also shown that lack of pain induced by palpation of the site of bone fracture often is a better indicator of full healing of the bone rather than simply relying on radiographic evidence of cortical bone union.^{18,27}

4.1. Current treatments and pharmacological therapies used to treat orthopedic-related pain

After joint replacement surgery or alignment and stabilization of a bone fracture, the most important factor in promoting bone healing and functional recovery is full and effective participation in physical therapy.⁶⁰ Loading and use of the joint and load-bearing bone are essential to minimize loss of bone and muscle mass, regain proprioceptive balance, promote integration of the implant, and healing of the fractured bone.²

Probably, the greatest impediment to effective participation in physical therapy after orthopedic surgery is skeletal pain, particularly as loading and moving the joint and bone are key aspects of physical therapy. Currently, 2 classes of pharmacological therapies are the mainstay of managing this pain, NSAIDs and opiates. These therapies are clearly efficacious and probably safe in young healthy individuals where bone healing occurs rapidly and effectively after orthopedic surgery or bone fracture. Although NSAIDs are quite effective in reducing musculoskeletal pain, recent data from rodents and retrospective studies in humans have shown that NSAIDs can inhibit bone formation and bone healing.^{1,25,57} While it remains unclear how much NSAIDs inhibition of prostaglandin production does reduce bone formation and healing in humans, recent data have shown that prostaglandin E2 is an integral part of the Wnt pathway involved in bone formation, and agonists of prostaglandin E4 have been shown to build bone in several species.⁷ Additionally, when overused, NSAIDs can have a variety of serious gastrointestinal, renal,

and hepatic toxicity that is estimated to result in >50,000 deaths per year in the United States alone.⁶ In light of the above issues with NSAIDs and bone healing, many believe that NSAID use is contraindicated in patients after joint replacement or bone fracture.^{26,59,67}

While it remains controversial whether opiates have any direct negative effect on bone healing, there is a growing consensus that long-term opiate use (>2-3 months) for chronic skeletal pain results in poor, if any, significant relief of skeletal pain,³¹ but it clearly is associated with loss of functional status, reduced likelihood to return to work, and a variety of issues related to dependence, depression, and diversion.^{3,39,68} In older patients (>60 years old), bone healing is remarkably slower than in young patients.²⁷ Even in younger patients with significant bone fractures and/or orthopedic surgery pain, full and effective bone healing can take months to years.¹⁶ As the major reason for using analgesics in combination with physical therapy is to rapidly improve the functional status of the patient, long-term use of NSAIDs and/or opiates carries with them significant unwanted side effects that may interfere with full and effective functional recovery.^{55,57,58}

4.2. Targeting nerve growth factor/TrkA for the relief of orthopedic surgery pain

In exploring possible alternatives to NSAIDs or opiates to control postoperative orthopedic surgery pain, we focused on the sequestration of NGF. Administration of anti-NGF before or after orthopedic surgery and after bone fracture reduced skeletal pain behaviors by 40% to 70% depending on the pain end point being assessed. Although anti-NGF clearly does not abolish skeletal pain following these procedures, it can be argued that complete relief of skeletal pain after orthopedic surgeries, such as those investigated here, would be unwanted because overloading and overuse of the bone could reinjure or re-fracture the bone.

Importantly, the issue of potential overuse of the injured or aged skeleton was recently raised in a human clinical trial of an anti-NGF (humanized anti-NGF monoclonal antibody). In these studies, while it was shown that anti-NGF therapy was efficacious in relieving pain due to osteoarthritis (OA)^{43,64} and low back pain,³⁸ a small but significant number of elderly (>65 years old) patients with moderate-to-severe OA pain on the highest dose of anti-NGF and an NSAID needed earlier-than-expected joint replacement.⁴³ As it was not clear whether this earlier-than-expected joint replacement was due to greater use of the diseased joint or the result of adverse effects on the bone tissue itself, a hold was placed on these clinical trials.¹⁰ What these data emphasize is that developing a better understanding of the effect anti-NGF may have either on overuse or whether it directly modulates cells in the skeleton will be very helpful in understanding the usefulness of anti-NGF and/or TrkA antagonists in treating postoperative orthopedic and bone fracture pain.

One area where progress has been made is in understanding the mechanisms by which blockade of NGF or its cognate receptor TrkA relieves skeletal pain. First, a very high proportion (>80%) of all sensory nerve fibers that innervate the bone and joint express and are modulated by TrkA.¹² Second, NGF has been shown to modulate the expression and function of a wide variety of molecules expressed by sensory neurons that innervate the bone and joint.^{50,62} Third, NGF may drive skeletal pain by inducing sprouting of TrkA+ sensory and sympathetic nerve fibers in the joint and bone.⁷¹ In the normal healthy joint and bone, the sensory and sympathetic innervation appears to be very tightly regulated so that

there are no sensory or sympathetic nerve fibers in the articular cartilage of the joint and few sensory or sympathetic nerve fibers in mineralized bone,^{12,63} both of which undergo significant and repeated mechanical loading and unloading. However, after injury to the joint or bone, the normally highly nonpermissive environment for the presence and sprouting of nerve fibers in articular cartilage and mineralized bone appears to be reduced,^{21,44,45,70} allowing sprouting¹³ into areas of the bone and joint that are normally poorly innervated by sensory and sympathetic nerve fibers.^{12,46} If rapid and effective repair of the injured joint or bone does not occur, NGF released from bone stromal cells seems to be able to drive both sensitization and sprouting of nerve fibers.^{8,36,51}

5. Conclusions and future studies

The major finding of this study is that when anti-NGF is given either before or after orthopedic surgery and after bone fracture, this therapy relieves skeletal pain by approximately 50%, which is similar to the pain relief obtained in human studies of advanced osteoarthritis pain. Limitations of the study are that we only examined 1 bone (the femur) in 1 gender (males) and in young adult mice. Clearly, developing a better understanding of orthopedic surgery and bone fracture pain and healing that occurs in young vs old, males vs females, and in other bones of the body will help in understanding which individuals will most benefit from specific mechanism-based analgesic therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative radiographs of a healing femur in a young adult (3 months old at the time of fracture) C3H mouse following a 3-point closed fracture procedure. A stainless steel pin is implanted into the intramedullary space of the femur 4 weeks before the mid-diaphyseal fracture. Callus formation is radiographically apparent by day 10. Cortical union becomes apparent at week 7,⁶⁶ and by week 11, palpation-induced pain behaviors return to baseline. Notice the intact patella throughout each time point, a key inclusion criterion.

Figure 2.

Anti-NGF treatment reduces the spontaneous nocifensive behaviors after orthopedic surgery and bone fracture in mice. (A) Time course for orthopedic surgery pain followed by fracture pain. Note that anti-NGF significantly reduces orthopedic surgery-induced skeletal pain by approximately 50% (B) and is effective at reducing moderate-to-severe pain as it reduces fracture pain by approximately 55% (C). Data are presented as mean $\pm$ SEM (* P < 0.05, t test, vs vehicle-treated mice figures [B and C]).

Figure 3.

Palpation as an indicator of bone fracture healing in pain-related behaviors. (A) On week 3 after fracture, palpation-induced nocifensive behaviors were significantly higher and remained elevated through week 9 when compared with spontaneous behaviors. (B) The number of rears after palpation was significantly lower on weeks 3, 7, and 9 when compared to spontaneous behaviors. By week 11, all spontaneous and postpalpation pain behavioral responses become equalized ($*P < 0.05$ vs spontaneous behavior). Note that palpation-induced pain behaviors continue after cortical union has occurred.

Figure 4.

Influence of patellar displacement on nocifensive behaviors after orthopedic surgery. While the surgical procedure to implant the stabilizing pin causes a short-lived orthopedic pain, if displacement of the patella occurs (from A to B and from C to D), surgical pain is significantly more severe and longer lasting ([E]: with patella displacement, as seen by the black squares) when compared to mice with intact patella position ([E]: no patella displacement, white triangles). Note that orthopedic surgical pain and radiographs need to be assessed in models of fracture pain to be able to differentiate the pain arising from surgical procedures, a displaced patella or bone fracture. Patella displacement most often occurs in the first 7 days after surgery when muscle tissues have not yet healed (* $P < 0.05$ vs naive, ** $P < 0.05$ vs no patella displacement).

Figure 5.

Group-housing mice aggravated fracture. Representative radiographs display examples of a femur with an initial simple fracture ([A] day 0) that became nonsimple ([A] day 7) when animals were group housed and an initial simple fracture ([B] day 0) that remained a simple fracture ([B] day 7) when animals were housed singly. Note that after fracture production, mice need to be housed singly, as 73% of simple fractures become nonsimple fractures within 7 days when animals are group housed (3 mice per cage) vs <10% of mice when singly housed (C). The fractures shown in panel (A) (day 7) meet the exclusion criteria for this study (* $P < 0.05$).

Table 1

Correlation between preclinical measurements using mice and clinical measures in humans for assessing fracture pain, functional status, and bone healing.

	<u>Mouse Measures</u>	<u>Human Measures</u>
<div><div>Fracture pain</div><div>Orthopedic pain</div></div>	Nocifensive behavior	Spontaneous (ongoing) pain
	Dynamic weight bearing	Ability to place weight on the affected limb
	Rears	Activity level and spontaneous use of the affected limb
	Weight	Weight gain or loss
	Palpation of the fracture site	Palpate fracture site to assess healed state
	Callus formation and resorption	Callus formation and resorption
	Cortical union	Cortical union