Published in final edited form as:

Brain Res Rev. 2009 April ; 60(1): 187–201. doi:10.1016/j.brainresrev.2008.12.012.

New advances in musculoskeletal pain

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Abstract

Non-malignant musculoskeletal pain is the most common clinical symptom that causes patients to seek medical attention and is a major cause of disability in the world. Musculoskeletal pain can arise from a variety of common conditions including osteoarthritis, rheumatoid arthritis, osteoporosis, surgery, low back pain and bone fracture. A major problem in designing new therapies to treat musculoskeletal pain is that the underlying mechanisms driving musculoskeletal pain are not well understood. This lack of knowledge is largely due to the scarcity of animal models that closely mirror the human condition which would allow the development of a mechanistic understanding and novel therapies to treat this pain. To begin to develop a mechanism-based understanding of the factors involved in generating musculoskeletal pain, in this review we present recent advances in preclinical models of osteoarthritis, post-surgical pain and bone fracture pain. The models discussed appear to offer an attractive platform for understanding the factors that drive this pain and the preclinical screening of novel therapies to treat musculoskeletal pain. Developing both an understanding of the mechanisms that drive persistent musculoskeletal pain and novel mechanism-based therapies to treat these unique pain states would address a major unmet clinical need and have significant clinical, economic and societal benefits.

Keywords

Bone healing; Osteoarthritis; Osteoporosis; Post-surgical pain; Fracture

1. Introduction

Musculoskeletal diseases and the associated musculoskeletal pain are major causes of disability in both the developed and developing world. Pain due to rheumatoid arthritis,

osteoarthritis, surgery-induced injury to the musculoskeletal system and bone fracture are common in our society. While mortality from these conditions is in general low, they have a major effect on disability, medical costs and patient quality of life which are in large part due to the associated musculoskeletal pain. As the average age of the population rises, the impact of musculoskeletal conditions on society will increase in parallel. Currently, there is a limited understanding of the mechanisms that drive musculoskeletal pain and a limited repertoire of analgesics available to treat musculoskeletal pain. In an effort to increase our understanding of the factors that drive musculoskeletal pain, and to develop platforms that can test the efficacy of novel analgesics, several new preclinical models of musculoskeletal pain have recently been developed. In the present review Julia Inglis and Susan Bove discuss preclinical modeling of osteoarthritis pain, Sarah Flatters discusses modeling postoperative pain in preclinical models and Patrick Mantyh discusses preclinical modeling of bone fracture pain. Developing an understanding of the mechanisms that drive different types of musculoskeletal pain and developing new analgesics to treat musculoskeletal pain would have significant clinical, economic and societal benefits.

2. Preclinical modeling of osteoarthritis pain: mechanisms and therapies

Osteoarthritis (OA) is the most common degenerative disease of joints, affecting at least 50% of people over 65 years of age, and occurring in younger individuals following joint injury. This figure is set to rise as OA prevalence increases with obesity, and as our aged population expands. Disease is characterized initially by cartilage degradation which precedes changes in the underlying subchondral bone. Although OA is regarded as a non-inflammatory disease, episodic synovitis does occur especially in late disease. Patients often present pain and disability following a significant loss of the articular cartilage. As there are currently no disease modifying agents, management of OA is by physiotherapy, pain relief, and ultimately surgical joint replacement.

Current analgesic agents such as paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDs) are only partially efficacious. NSAIDs have increased morbidity in this elderly population due to associated gastric ulceration and cardiovascular complications. Even with current therapies the quality of life in the majority of patients with OA is significantly impaired by severe, often intractable, pain. Preclinically, a range of animal models are used for the study of OA progression and for evaluating the efficacy of drugs (Pritzker, 1994). Until recently, mechanisms of pain generation in OA have been difficult to study due to a lack of clinically relevant animal models, as well as sensitive methods for measuring pain behavior. This review will focus on rodent models of OA pain, their relevance to human disease, and insights into OA pain mechanisms.

2.1 Rat models of OA pain

A single intra-articular injection of monosodium iodoacetate (MIA) into the femorotibial joint results in chondrocyte death and the development of an OA resembling human disease (Guzman et al., 2003). Monosodium iodoacetate inhibits glyceraldehyde-3-phosphate dehydrogenase activity in chondrocytes, resulting in disruption of glycolysis and eventual cell death. It is reported that at low doses MIA selectively damages articular cells, however, MIA is a non-specific alkylating agent which is capable of inducing large-scale cellular

death when administered at high concentrations, therefore caution is warranted when utilizing large amounts of MIA to initiate experimental OA (Pritzker, 1994; Barve et al., 2007). The specificity of this method of OA development has been questioned in a recent study by Barve et al. (2007) in which they demonstrated significant transcriptional differences between this model and human OA. It appears that little similarity exists at the transcriptional level during the development of MIA-induced OA when compared to the long-term progression and development of OA in humans (Barve et al., 2007). This is not surprising as the MIA model is a chemically-induced, rapidly progressive model and this may indicate that this model is more useful for discerning therapeutic approaches for OA pain intervention rather than for disease modification. To date, the rat MIA model is the best characterized animal model for OA pain. Standard analgesics, including NSAIDs and COX2 inhibitors are effective in reducing pain in this model suggesting an important contribution from prostaglandins (Bove et al., 2003; Pomonis et al., 2005), however the effect of NSAIDs may be dependent on the dose of MIA used and the time point tested (Ivanavicius et al., 2007). At lower doses of MIA (1 mg) NSAIDs appear to retain single dose effectiveness out to 2 weeks post MIA injection when animals were assessed for changes in hind paw weight distribution (Bove et al., 2003; Pomonis et al., 2005). When MIA is administered at higher doses (2–3 mg/joint) and/or later time points post MIA injection (>3 weeks) are assessed, acute efficacy of NSAIDs is more difficult to achieve. Consistent results have only been obtained with chronic dosing of NSAIDs or acute administration of opiates and gabapentin (Bove personal observation; Pomonis et al., 2003; Fernihough et al., 2004; Ivanavicius et al., 2007). These observations, combined with recent findings in this model indicating an increase in TRPV1 and CGRP expression in the nerves innervating the diseased joint and a substantial induction of ATF-3 expression in the L5 dorsal root ganglion (DRG), clearly point to the development of a neuropathic component to the disease (Fernihough et al., 2005; Ivanavicius et al., 2007). The development of peripheral and central sensitization in human OA is not uncommon and typically occurs during later stages of the disease (Bajaj et al., 2001; Bradley et al., 2004). This secondary mechanical allodynia, or referred pain, develops in the MIA model as well and can be reversed with compounds and drugs designed to target neuropathic pain, such as opiates and gabapentin, providing further support for the utility of the MIA model for testing of compounds for therapeutic intervention in OA (Combe et al., 2004; Fernihough et al., 2004; Beyreuther et al., 2007). In addition to pharmacological characterization of this model, a host of investigators have started to utilize other technologies to further assess the potential translatability of this model to the clinic, including image analysis using MRI, μ CT and ultrasound, electrophysiology studies, and gait analysis (Morenko et al., 2004; Schuelert and McDougall, 2006, 2008).

A second model that has recently been characterized for its utility as a tool for developing pharmacologic interventions for the treatment of OA pain is the rat medial meniscal tear (MMT) model (Bove et al., 2006). This model was first described by Bendele (2001) as a surgically-induced rapidly progressive (<3 weeks) model in which to test agents for disease modification of OA. The MMT model is initiated by transecting the medial collateral ligament of the femorotibial joint to expose the joint space. A full cut of the medial meniscus is then made at its narrowest point in order to simulate a complete meniscal tear. Histologically, the MMT model presents similarly to human OA with cartilage damage

starting with fibrillation and loss of proteoglycan, progressing to complete loss of the cartilage layer and exposure of the subchondral bone (Bendele, 2001). Subchondral bone changes can be noted even prior to the complete loss of the cartilage layer and large medial tibial plateau osteophytes are also described in this model. Symptomatically, the MMT model has been shown to demonstrate both nociceptive (alterations in hind paw weight distribution) and referred (secondary mechanical allodynia using von Frey monofilament hairs) types of pain and is amenable to pharmacologic intervention (Bendele, 2001). Similar to the MIA model, NSAIDs, COX2 inhibitors and acetaminophen are all efficacious against nociceptive pain in the MMT model whereas gabapentin effectively reverses referred pain. Two novel compounds for OA pain have been tested in this model in our laboratory, the selective mGluR5 inhibitor, MPEP, and the peripheral benzodiazepine receptor ligand, PK-11195. These compounds work well to reverse nociceptive pain, but have not demonstrated efficacy for referred pain in the MMT model (Bove unpublished observations).

2.2. Mouse models of OA pain

With the expanding availability and use of transgenic mice, it is becoming increasingly important to have clinically relevant validated murine models of OA pain. Mouse models are commonly used to study disease pathogenesis, but to date little has been reported on pain in these models. Mouse models can be categorized as spontaneous (i.e. based on a genetic predisposition), and experimentally induced through the injection of chemicals into the knee, or surgical induction of joint damage (for review see Brandt, 2002).

Genetic predisposition is thought to contribute to between 38–65% of OA (Helminen et al., 2002). The most widely studied spontaneous murine model of OA is the STR/ort mouse, which has an as yet unknown genetic deficit. Though useful to study pathogenesis, polyarthritic mice are difficult to study using standard pain assessment, such as weight bearing. OA can be modeled in the mouse by induction of joint instability, through collagenase, or papain injection, and chemical induction of chondrocyte death, through the injection of iodoacetate. These models resemble disease in humans, with cartilage erosion, thickening of the subchondral bone and osteophyte formation. However, as discussed above, the relevance of these models to the etiology of human OA is questionable, and specificity is likely to be dose-dependent.

Neuronal sensitization in OA has been investigated in the STR/1N mouse (related to the STR/ort mouse) and in animals with collagenase-induced OA (Averbeck et al., 2004). It was shown that mice with OA have increased spontaneous, and bradykinin-induced PGE2 production from isolated joints from arthritic animals, where as no change in spontaneous, or evoked CGRP release was observed. As PGE2 is a key hyperalgesic agent, the authors hypothesized that the prostaglandin may be a key mediator in chronic pain associated with OA. However, no pain behavior, or use of analgesics (such as NSAIDs) has been reported in these models to further these observations.

The first murine surgical model developed was transection of the medial collateral ligament, and removal of the cranial half of the medial meniscus (Clements et al., 2003). This results in joint instability, and the development of a severe OA within 4 weeks, with many features

resembling human OA, such as chondrocyte death, cartilage erosion, and cartilage neo-epitope formation. More recently, a more mild surgical induction of joint instability has been developed (Glasson et al., 2004). In this model, transection of the medial meniscotibial ligament is performed, resulting in destabilization of the medial meniscus (DMM), and the development of OA in around 8 weeks (Glasson et al., 2005). This may resemble a mild injury an individual may develop, that may precipitate OA, and has little or no synovitis associated with disease progression and no neuropathic component as assessed by ATF-3 expression (Inglis et al., 2008). DMM has recently been characterized as a model of chronic OA pain (Inglis et al., 2008). As in the clinic, morphine is analgesic in DMM, as are COX2 inhibitors. Unlike other models of OA, pain only occurs late in disease, characterized by both changes in weight bearing, and spontaneous locomotor activity. This is a kin to human disease in which many patients present with severe damage, and up to 40% of patients with radiological evidence of significant joint degeneration are asymptomatic (Kidd, 2006). Administration of the non-CNS penetrant μ -opioid receptor antagonist naloxone methiodide unmasks pain 4 weeks earlier in mice with DMM. It has been shown that this was due to a transient increase in μ -opioid receptor expression in the peripheral nerve resulting in an inhibition of pain at early stages of disease (Inglis et al., 2008). This highlights that both pro- and anti-algesic mechanisms occur during arthritis, and the balance of these may determine the extent of pain experienced. The mechanisms by which OA develops from an asymptomatic condition to a painful disease remain to be established.

2.3. Conclusions

In recent years a range of rodent models of OA pain have been developed which model some, or all aspects of the human condition. MIA in the rat is the best characterized model of OA pain, and provides a rapid and reliable model for assessment of potential therapeutics. In contrast, surgical models of disease have a longer lag time before the onset of pain, but may have similar etiology to secondary OA in humans. These models may prove useful in investigating the transition of asymptomatic to painful OA. Prostaglandins appear to be key mediators in the development of all the above models, and in the clinic with the efficacy of NSAIDs and COX2 inhibitors. Though progress has been made, little is understood in how pain occurs in OA, and its similarities and differences to other pain states, such as inflammatory and neuropathic pain. Future challenges will include identifying the site of the pain in the OA joint, as there is a poor correlation between histological and radiological joint damage and pain (McDougall, 2006). The use of animal models will be vital for the investigation of changes in disease as clinical samples of these areas are unavailable. Through an increased understanding of the development of pain in OA models, and its relationship with the degenerative process, novel therapies may be developed to treat this widespread condition.

3. Modeling postoperative pain in the laboratory

Persistent postoperative pain occurs following various common surgical procedures including thoracotomy, coronary artery bypass surgery, inguinal hernia repair and caesarean section. The estimated incidence of persistent postoperative pain following these surgeries is 30–40%, 30–50%, 10% and 10%, respectively (Kehlet et al., 2006). Furthermore an

estimated 2– 10% of these surgical patients will classify their pain as severe i.e. scoring 6 – 10 on a visual analogue scale, where 10= worst pain imaginable (Kehlet et al., 2006). The significance of these incidences is evident when the procedures is considered; in 1996, over 2 million people in the US alone underwent either coronary artery bypass, inguinal hernia repair or caesarean section surgeries (Owings and Kozak, 1998). Therefore persistent postoperative pain is a wide-spread clinical problem with a significant impact on quality of life.

A variety of surgical techniques have been employed (primarily in rats) to model postoperative pain. These models can be broadly divided into two categories; incision models — where tissue is cut and then sutured, and retraction models — where tissue is cut, retracted for a period of time and then sutured. Table 1 summarizes the pain behaviors displayed by incision and retraction models of postoperative pain.

3.1. Incision models of postoperative pain

Over a decade ago the first incision model of postoperative pain was described (Brennan et al., 1996). In anaesthetized rats, a 1 cm incision through the skin, fascia and muscle of the plantar hindpaw was performed and then closed with two nylon sutures. This surgical procedure evoked significant mechanical hypersensitivity (assessed with von Frey stimulation) at the surgical site for 3–5 days postoperatively (Brennan et al., 1996; Zahn and Brennan, 1999). A secondary mechanical hypersensitivity was also observed for 2–3 days postoperatively in this model, when von Frey stimulation was performed on the plantar hindpaw 10 mm distal to the surgical site (Brennan et al., 1996; Zahn and Brennan, 1999). Postoperative heat hyperalgesia is also present in this model at the surgical site, but not at the distal site, and this lasts for 7 days post surgery (Zahn and Brennan, 1999). In comparison to this model involving an incision in glabrous skin, another incision model of postoperative pain was developed that employed an incision in hairy skin (Duarte et al., 2005). In anaesthetized rats, a 1 cm incision was made in the hairy skin of the rat's back (avoiding fascia and muscle) and then closed with one silk suture. This surgery evoked hypersensitivity to von Frey stimulation at 0.5 cm and 1 cm sites from the incision for 1 week postoperatively. A secondary mechanical hypersensitivity was also evident for 3 days postoperatively using von Frey testing 2 cm from the incision (Duarte et al., 2005). An incision model of postoperative pain was also developed that focused on secondary hyperalgesia (Pogatzki et al., 2002). In anaesthetized rats, a 3 cm skin/fascia incision was made in the midportion of the posterior hindlimb, the underlying gastrocnemius muscle was also split and then the skin closed with three nylon sutures. This surgery evoked secondary hypersensitivity to von Frey stimulation on the plantar hindpaw for 8 days postoperatively. Radiant heat stimulation of the plantar hindpaw demonstrated a lack of secondary heat hyperalgesia in this model (Pogatzki et al., 2002).

A disadvantage with these incision models is their limited time course of evoked pain behaviors. Thoracotomy, coronary artery bypass surgery, inguinal hernia repair and caesarean section surgeries all involve essential and often prolonged tissue retraction that could account for the persistent nature and relatively high incidence of pain following such

surgeries. However, the incision models do not accurately reflect this clinical scenario of postoperative pain, i.e. prolonged tissue retraction resulting in persistent pain.

3.2. Retraction models of postoperative pain

In the last few years, two models of postoperative pain (Buvanendran et al., 2004; Flatters, 2008) have been developed which evoke pain through more invasive and prolonged means than the incision models. Firstly, the thoracotomy procedure was replicated in anaesthetized rats (Buvanendran et al., 2004). This involved incisions in skin, intercostal muscle and pleura between the 4th and 5th ribs. A retractor was then placed in the surgical site to retract the ribs by 8 mm for 1 h. The muscles and skin were then closed with silk and nylon sutures, respectively. This thoracotomy surgery evoked mechanical allodynia (assessed with von Frey stimulation adjacent to at the surgical site) and cold allodynia (assessed by responses to acetone application). Both these pain behaviors lasted for at least 40 days postoperatively. Interestingly, only 50% of rats developed pain behaviors following the thoracotomy procedure and this correlated with the presence of degeneration in the 4th intercostal nerve. Degeneration was caused by the retractor compressing the nerve against the rib which is often unavoidable due to the location of this nerve. Rats who did not display allodynia following thoracotomy also lacked signs of degeneration in this nerve (Buvanendran et al., 2004). This suggests that the pain behaviors evoked by thoracotomy have a neuropathic origin.

Most recently a new rat model of persistent postoperative pain was designed to combine both prolonged tissue retraction (akin to clinical procedures) and the evaluation of pain behaviors via hindpaw stimulation (Flatters, 2008). The skin/muscle incision and retraction (SMIR) surgery involves incisions in the skin and superficial muscle of the medial thigh. Following blunt dissection, a micro-dissecting retractor was placed in the surgical site to retract the skin and muscle for 1 h. The muscles and skin were then closed with silk and Vicryl® sutures, respectively. SMIR surgery evoked 3 weeks of mechanical hypersensitivity (assessed with von Frey stimulation) in the plantar ipsilateral hind-paw. SMIR surgery did not evoke either heat hyperalgesia or cold allodynia in the plantar ipsilateral hindpaw. The saphenous nerve is potentially stretched during SMIR surgery due to its superficial location on the medial thigh. However, in contrast with the thoracotomy model there were no signs of degeneration in the saphenous nerve at, or proximally or distally to, the surgical site. In addition, very little to no degeneration was detected with ATF3 staining in the dorsal root ganglia from SMIR-operated rats. These data suggest that prolonged retraction of superficial tissue can evoke a persistent pain syndrome that has a non-neuropathic origin.

3.3. Conclusions

Persistent postoperative pain is a relatively frequent occurrence due to the large numbers of patients undergoing routine surgeries that involve essential incision and retraction of tissues. Great progress has been made in understanding the mechanisms underlying acute pain caused by incision with the use rat models of skin incision. New advances in modeling of postoperative pain have been made with models that involve prolonged tissue retraction resulting in persistent pain. Hopefully, new insights can be gained into the mechanisms responsible for persistent postoperative pain with the use of these new models. Enhanced

understanding of such mechanisms will aid the development of new analgesic therapies for the prevention and treatment of persistent postoperative pain.

4. Fracture pain: causes, consequences and therapeutic opportunities

4.1. The clinical problem of bone fracture and fracture pain

Fracture pain is a common form of acute and chronic pain in both the young and the old (Yates and Smith, 1994; Moholkar and Ziran, 2006). In young individuals (<30 years old) the majority of fractures are due to sports and motor vehicle related accidents (Court-Brown and Koval, 2006). More recently, with the increase in armed conflicts along with the development of body armour, Improvised Exploding Devices (IEDs) and landmines, a significant number of mostly young adults now survive injuries that previously would have been fatal (Owens et al., 2007). However, these blast-induced injuries often produce severe, complex, wide-spread and highly painful fractures of the head and extremities (Owens et al., 2007). Although young males have historically had a higher incidence of fractures than young females (Koval and Cooley, 2006), with the increasing number of women participating in sports (Hame et al., 2004) and military roles (Feuerstein et al., 1997), this gender difference in fracture incidence in the young is declining.

As humans age there is an increased risk of fracture due to age-related bone loss (Newton-John and Morgan, 1970; Mazess, 1982). Thus, 50% of women, and 25% of men, over 50 years old will have an age-related fracture sometime in their lifetime (Dennison et al., 2006). In the elderly, not only are fractures more frequent (Newton-John and Morgan, 1970; Mazess, 1982) but bone healing times are also significantly increased (Nilsson and Edwards, 1969). Following fracture of a weight bearing bone such as the vertebrae, hip or femur, adequate control of fracture pain is essential for the patient to be able to robustly and effectively participate in rehabilitation (Walker, 2004). A delay in rehabilitation may result in loss of both bone and muscle mass which, in the elderly, can result in significant morbidity and mortality (Center et al., 1999; Johnell and Kanis, 2005, 2006). As individuals live longer, throughout the world age-related fractures and associated fracture pain have become an increasing medical, social and economic problem. Thus, the US Surgeon General has recently estimated that by 2020 one in every two Americans over the age of 50 will be at risk from osteoporosis or low bone mass fractures (The Surgeon's General report, 2004). Currently, only 50% of individuals over 60 years old who have a osteoporotic hip fracture (that in 95% of the cases is actually fracture of the neck of the femur) will ever regain their pre-fracture status as judged by the ability to walk, and the need for aides at home (The Surgeon's General report, 2004). A major problem for patients with fractures of load bearing bones is the lack of analgesics that can control fracture pain without CNS side effects that make the patient less likely to participate in rehabilitation, more likely to fall (opiates) or inhibit bone healing (NSAIDs). This lack of the ability to be physically active results in a dramatic decline in the health of the patient so that 20% of individuals who suffer an age-related hip fracture die within a year of fracture. An additional 20% of individuals with hip fracture end up in a nursing home within a year of the fracture and will never return to living independently (Cooper et al., 1992). In 2000 there were an estimated 9 million osteoporotic fractures and, with the increase in the aging population, this number is expected to reach 22

million by 2050. Worldwide, medical costs directly and indirectly attributed to osteoporotic fractures were \$131 billion in 1990. With increases in the aging population these costs are expected to rise three fold by 2050 (Cooper et al., 1992).

4.2. Use of bone is required for bone healing and maintenance of skeletal health

Load bearing bones are unique in that they truly are “use it or lose it” organs. As such, a load bearing bone is constantly being remodeled over the entire lifespan of the individual in response to loading and use. This is exemplified by how rapidly bone is lost even in young, healthy subjects in a zero gravity environment. Following 1–2 months in a zero gravity environment young individuals in the peak of health show a remarkable loss of bone mineral density, bone strength and a marked predisposition to bone fracture (Lang, 2006; Payne et al., 2007). While it remains unclear how bone senses mechanical loading and why bone is lost so rapidly when not used, what is clear is that this “use it or lose it” property of bone can have a major and life altering effect on both young and old who suffer from painful fractures of a load bearing bone (Zuckerman et al., 1990; Koval et al., 1998; Brumback et al., 1999; Ekstrom and Elmstahl, 2006). Unlike skin, which following injury heals best when not mechanically stressed, for bone to heal most rapidly and effectively requires initial stabilization of the fracture site followed by rehabilitation that requires next day use and loading of the fractured bone (Zuckerman et al., 1990; Koval et al., 1998; Brumback et al., 1999; Brunton et al., 2005). It needs to be emphasized that control of fracture pain is important following initial fracture (days to weeks) as at this time patients have not yet lost the bone or muscle mass required for ambulation, loading of the bone and rehabilitation (Brumback et al., 1999; Emami et al., 2001). Thus, in young patients with complex or severe fractures (usually the result of motor vehicle, sports and military related injuries) (Court-Brown and Koval, 2006; Owens et al., 2007) or in older patients with age-related fractures (Orsini et al., 2005), (usually the result of osteopenia/osteoporosis) early and effective rehabilitation greatly increases the likelihood of successful healing and functional recovery of the fractured, load bearing bone.

4.3. Current therapies available to treat fracture pain

The greatest impediment to having both young and old fracture patients participate in early use and loading of their fractured bone(s) is the lack of available analgesics that can control fracture pain without unwanted CNS or bone healing side effects. Currently, the treatment of pain following skeletal fracture of a load bearing bone involves stabilization of the fractured bone, bed rest only if absolutely required and the use of NSAIDs and opiates (Yates and Smith, 1994; Ekman and Koman, 2005; Greenfield, 2006; Mamaril et al., 2007). Whereas NSAIDs are effective in reducing a variety of musculoskeletal pains (Balano, 1996; Mason et al., 2004), there are reports suggesting that these agents either directly or indirectly inhibit fracture healing in mice (Murnaghan et al., 2006), rats (Simon and O'Connor, 2007) and humans (Giannoudis et al., 2000). Thus, studies in rodents have suggested that NSAIDs and selective cyclo-oxygenase-2 (COX-2) inhibitors retard callus formation and effective bridging of the fracture site which results in delayed bone healing, increased incidence of fracture nonunion and decreased bone strength (Simon et al., 2002; Gerstenfeld et al., 2003; chLeonelli et al., 2006). These findings have been supported by some (Bhattacharyya et al., 2005; Koester and Spindler, 2006) but not all studies in humans (Wheeler and Batt, 2005).

Whereas the extent to which NSAIDs inhibit bone healing in humans remains contentious, what is clear is that many orthopedic surgeons believe that NSAIDs are contraindicated in patients undergoing fracture healing (Varghese et al., 1998; Aspenberg, 2002).

Although opiates are commonly used to control severe fracture pain (McCann and Stanitski, 2004; Ekman and Koman, 2005; Mahowald et al., 2005; Mamaril et al., 2007), there are no definitive or well controlled studies in any species that have examined the direct effect that sustained administration of opioid agonists have on bone injury/repair/healing. In studies that have examined the effects that opiate agonists and antagonists have on malignant bone remodeling and soft tissue repair the results are controversial. Thus, whereas some studies suggest that opiate agonists stimulate bone and tissue healing (Poonawala et al., 2005) and opiate antagonists accelerate bone and tissue healing (Petrizzi et al., 2007; Zagon et al., 2007) other studies suggest the opposite (King et al., 2007). However, one area where there is nearly complete agreement is that opioids have a variety of CNS side effects that can indirectly inhibit bone healing. Opioids as a class cause increased somnolence, agitation, constipation, dizziness and cognitive impairment which can reduce mobility and the ability to participate in physical rehabilitation which results in loss of bone and / or muscle mass which further results in delayed bone healing (Ensrud et al., 2003). In young individuals with severe fractures, long-term opiate use can result in dependence and a reduced ability to promptly and fully participate in effective rehabilitation that is necessary for early and effective bone healing (Mahowald et al., 2005; Massey et al., 2005). In elderly patients, opioid-induced CNS side effects tend to be more pronounced (Feinberg, 2000). For example, a recent clinical study in humans demonstrated a significant increase in risk of bone fracture in users of morphine and other mu opiates (Vestergaard et al., 2006). Although a conclusion as to why patients taking opiates were more likely to experience a fracture was not reached, the authors speculated a possible increase in the risk of falls was due to opiate induced dizziness and loss of balance. Thus, while NSAIDs and opiates are clearly effective in attenuating fracture pain, their side effect profile can interfere with fracture healing. This makes the development of novel, mechanism-based therapies to treat fracture pain that lack CNS and bone healing side effects a clear priority for both young and old patients with bone fracture.

4.4. Preclinical models of fracture pain

Our current lack of therapies to treat fracture pain is in large part due to the lack of animal models that closely mirror human fracture pain. Recent studies have shown that mineralized bone, marrow and periosteum (the thin fibrous sheath that surrounds the outer surface of mineralized bone) all receive a significant innervation by primary afferent sensory neurons (Fig. 1) and post-ganglionic sympathetic neurons (Hill and Elde, 1991; Hukkanen et al., 1993; Mach et al., 2002). What we do not know are the mechanisms by which sensory neurons that innervate the skeleton are activated even in common conditions such as osteoarthritis, low back pain or fracture. Recently, preclinical animal models of fracture pain (Fig. 2) were developed in the rat and mouse (Koewler et al., 2007; Freeman et al., 2008; Jimenez-Andrade et al., 2008). These models of bone fracture pain were adapted from the rodent closed femur fracture model that has been extensively employed to explore the effects of bone morphogenic proteins (Chhabra et al., 2005), NSAIDs (Gerstenfeld et al.,

2003) and selective COX-2 inhibitors (Simon et al., 2002) on bone remodeling and bone healing. A key advantage of the bone fracture pain model is the ability to examine simultaneously the effects of a potential analgesic on bone pain and bone healing following fracture. Concerning skeletal pain, the models utilized here appear to closely mirror the sequence of events observed in humans with significant skeletal pain due to fracture of a load bearing bone. In these models, fracture induces pain-related behaviors (guarding, flinching, activity, and reduced ability of weight bearing on the fracture bone) that are exacerbated by movement of the fractured bone (Fig. 3). These fracture-induced pain behaviors peak at 2 days post-fracture and, as in humans, decline with soft callous formation around the fracture site, bone stabilization/ fixation, bridging of the fracture site and bone healing (Blasier, 2000; Santy and Mackintosh, 2001; Pape and Giannoudis, 2006). Similar to humans (Bone et al., 2004), the pain-related behaviors in the mouse are reversed by morphine and ultimately resolve if appropriate bone healing occurs.

4.5. Mechanisms that drive fracture pain

A major and yet unanswered question is whether the pain that immediately follows fracture is driven primarily by mechanical or chemical transducers expressed by nociceptors that innervate the bone. In attempting to address this question, it is instructive to examine the timecourse, severity and factors that can stimulate or attenuate fracture pain in humans. The initial pain that follows acute fracture of the human femur is most frequently described as stabbing, aching and very intense. For example, patients often refer to femoral fracture pain as the worst pain that they have ever felt in their life (Santy and Mackintosh, 2001; Crandall et al., 2002). Interestingly, this acute pain is attenuated by resetting and stabilizing the mineralized bone/apposed periosteum (Fig. 4) at the site of fracture and this intense pain can be reactivated within seconds by movement and mechanical distortion of the fractured femur (McSwain, 1992; Hedequist et al., 1999; Santy and Mackintosh, 2001; Bone et al., 2004; Camuso, 2006). This clinical picture would suggest that mechanosensitive nerve fibers including those present in the densely innervated periosteum, are pivotally involved in driving the initial fracture pain. Additional support that distortion of mechanosensitive nerve fibers located in the periosteum are involved in generating initial fracture pain is suggested by experimental studies performed in human volunteers. Direct mechanical stimulation (induced by scratching with a point of a needle or drilling with a Mathews wire) of the periosteum in awake humans elicited an immediate sharp arresting pain that possessed the lowest threshold as compared to the ligaments, the fibrous capsule of the joints, tendons, fascia and finally the least sensitive, muscle (Inman and Saunders, 1944).

While we do not yet know the exact mechanisms that drive fracture pain, our working hypothesis, gleaned from clinical and basic science literature, is that there are at least five distinct but overlapping events that drive this pain (Jimenez-Andrade et al., 2007; Koewler et al., 2007; Freeman et al., 2008). First, within seconds of acute fracture, mechanotransducers expressed by nociceptors that innervate the bone are directly activated by mechanical distortion of the periosteum and the underlying mineralized bone. Second, within minutes to hours of the initial fracture there is a marked influx of hematological and inflammatory cells into the fracture site which results in activation of nociceptors that express receptors for cytokines, chemokines and inflammatory factors such as bradykinin,

nerve growth factor or prostaglandins that are frequently released upon tissue injury (Hukkanen et al., 1993; Levine et al., 1993; Haegerstam, 2001). Third, these factors may directly excite, as well as sensitize, and induce sprouting of mechanosensitive nociceptors in the bone and newly formed callus so that normally non-noxious mechanical stimulation of bone or callus is now perceived as painful. Fourth, if there is continuing discharge of nociceptors innervating the fractured bone this could induce a central sensitization characterized by neurochemical and cellular changes in the dorsal horn of the spinal cord and higher brain centers that facilitate the transmission and perception of pain. In the case where the fracture does not produce severe nerve injury and proper bone healing occurs, changes in the peripheral and CNS would return to a baseline state and the hyperalgesia and allodynia of the formerly fractured bone will disappear. However, in cases where significant nerve injury occurs following fracture, the peripheral and central sensitization may be maintained and be accompanied by a fifth change, the inappropriate sprouting and interaction of sensory and sympathetic neurons that innervate the bone. These changes may contribute to a component of the chronic pain observed in individuals with complex regional pain syndrome (CRPS); in approximately 45% of patients with CRPS, fracture is the most common precipitating event (Sandroni et al., 2003; de Mos et al., 2006).

4.6. Understanding and developing mechanism-based therapies to treat fracture pain

To test whether the rodent fracture pain model could indeed serve as a platform for examining new therapies to potentially treat fracture pain we focused on a novel therapy involving an anti-NGF antibody sequestering approach. Previously, it has been reported that a variety of inflammatory, immune and stromal cells up-regulate the expression of NGF and NGF receptors following fracture (Grills and Schuijers, 1998) and tissue injury (McMahon et al., 2006). In the adult, NGF can directly activate and sensitize sensory neurons involved in the conduction of pain originating from the skin (Lewin et al., 1993; Lewin et al., 1994; Bennett et al., 1998) and visceral organs (Dmitrieva and McMahon, 1996; Delafoy et al., 2003). NGF is thought to excite and sensitize sensory neurons by binding to its cognate receptors trkA and p75 that are expressed by a subpopulation of mostly unmyelinated and thinly myelinated sensory neurons (Hefti et al., 2006; Pezet and McMahon, 2006). NGF binding to trkA has been shown to directly depolarize trkA-expressing nociceptors *in vivo* and *in vitro*. Binding of NGF to trkA also directly lowers the threshold for depolarization in these neurons (Kerr et al., 2001; Shu and Mendell, 2001; Hefti et al., 2006). Additionally, NGF has been shown to modulate and/or sensitize a variety of neurotransmitters, receptors, ion channels and structural molecules expressed by nociceptors including: neurotransmitters (substance P, brain derived neurotrophic factor and CGRP), receptors (bradykinin, P2×3), channels (TRPV1, ASIC-3 and sodium channels), transcription factors (ATF-3), and structural molecules (neurofilaments and the sodium channel anchoring molecule p11) (Gould et al., 2000; Ji et al., 2002; Bielefeldt et al., 2003; Hefti et al., 2006; Pezet and McMahon, 2006). It has also been shown that NGF lowers the threshold and enhances the response of nociceptors to mechanical stimuli (Malik-Hall et al., 2005), suggesting that NGF may play a role in activating/sensitizing mechanotransducers in sensory fibers that innervate bone. Together, these results suggest that NGF may be an upstream regulator of a variety of neurotransmitter/receptors that are expressed by nociceptors that innervate the bone and that are involved in sensing and transmitting pain following fracture of the skeleton.

Using a mouse model of fracture pain and bone healing we demonstrated that following femoral fracture anti-NGF therapy attenuated fracture-induced pain-related behaviors while having no observable CNS side effects and either no effect or a positive effect on measures of bone healing (Fig. 5). Concerning the timing of the administration of anti-NGF therapy, it should be emphasized that anti-NGF was first administered 1 day post-fracture, which is a time point when a human would begin to receive long-term analgesic therapy for fracture pain. The analgesic effect of anti-NGF therapy in reducing skeletal pain was statistically significant at the first time point examined (which was day 2 post-fracture) and the analgesic efficacy of the therapy remained as long as pain-related behaviors were present. Importantly, the analgesic effect of anti-NGF did not result in inappropriate loading of the fractured bones as assessed by the number of spontaneous vertical stands that require mechanical loading of the two hind limbs. Additionally, anti-NGF therapy did not result in longer healing times that might occur if there is inappropriate loading or stress on the fractured bone (Kenwright and Gardner, 1998; Gardner et al., 2006).

Together, the above data suggests that these rodent fracture pain models have the potential to allow investigators to begin to define the mechanisms that generate and maintain fracture pain as well as serving as a platform to test the efficacy and side effect profile of novel analgesics. One hypothesis concerning the sensory innervation of bone, that may partially explain why anti-NGF therapy is effective in attenuating skeletal pain, is that the majority of nociceptive C-nerve fibers that innervate the bone are CGRP/trkA-expressing fibers, with few if any IB4/RET+ sensory nerve fibers being present in bone. Previous studies have shown that CGRP/trkA+ primary afferent sensory neurons innervate the human, (Ozawa et al., 2006), and rodent intervertebral discs (Ozawa et al., 2003), and rat and mouse femur (Bjurholm et al., 1988; Hill and Elde, 1988; Artico et al., 2002; Mach et al., 2002), and the rat skull, whereas relatively few unmyelinated non-peptidergic IB4/RET+ nerve fibers have been observed innervating the femur and rat skull (Mach et al., 2002). Whether bone is indeed selectively innervated by CGRP/trkA+ but not IB4/RET+ nerve fibers, or whether the inability to detect IB4/RET+ nerve fibers in bone is due to technical and methodological issues, remains to be definitively resolved. However, if bone is selectively innervated by CGRP/trkA+ sensory nerve fibers this might present a unique therapeutic opportunity as bone would lack the redundancy of having both peptidergic CGRP/trkA+ and non-peptidergic IB4/RET+ sensory nerve fibers which both richly innervate the skin. Thus, a therapy that specifically targets the nociceptors that express CGRP/trkA+ may be more efficacious in relieving bone pain where there is really only one major class of nociceptors vs. skin which receives a significant innervation by two distinct classes of nociceptors.

4.7. Conclusions

Currently, there is a significant unmet clinical need to develop novel analgesics to treat fracture pain that do not have unwanted CNS and/or bone healing side effects. Thus, in both the young and old, females and males, optimal bone healing requires the patient to use and load the fractured bone as soon as possible following initial stabilization of the fracture and throughout the entire rehabilitation regimen. The most common reason that patients do not fully and effectively participate in this needed rehabilitation is the lack of effective analgesics that do not have unwanted bone healing or CNS side effect. If fracture pain is not

well controlled, the necessary bone loading and rehabilitation will be delayed (or not occur at all) resulting in loss of bone and muscle mass, lack of appropriate bone healing, loss of mobility with an ensuing increase in morbidity and mortality. Recently, the first preclinical animal models of fracture pain have been developed. These models have the potential to allow investigators to define the mechanisms that generate and maintain fracture pain and serve as a platform to test the efficacy and side effect profile of novel analgesics.

Developing an understanding of the mechanisms that drive fracture pain and broadening the availability of analgesics that allow the patient to use and mechanically load the fracture bone throughout the early, middle and late physical rehabilitation process would address a major unmet clinical need and have significant clinical, economic and societal benefits.

Acknowledgments

SJLF is supported by a Capacity Building Award in Integrative Mammalian Biology funded by the BBSRC, BPS, HEFCE, KTN and MRC.

JJI is supported by the Arthritis Research Campaign.

PWM's research is funded by NS23970 and NS048021 from the National Institutes of Health and by a Veteran's Administration Merit Review.

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Fig. 1.

Sensory innervation of the mouse bone. A μ CT 3D image of a mouse femur illustrating the areas used for analysis of bone innervation (A). Confocal photomicrograph showing CGRP in the mouse femur (B). Low power photomicrograph of the proximal head of the mouse femur where the CGRP-positive (+) fibers are bright white and are present in the marrow and surround the trabeculae (white arrowhead). The inset in the top right of (B) shows the average diameter of individual fibers in a bundle of CGRP fibers found in the marrow. High power photomicrographs of CGRP expressing fiber in the marrow (C) and periosteum (D). Note that the CGRP+ nerve fibers are in close proximity to blood vessels within the Haversian canal system, while in the periosteum CGRP+ nerve fibers form a dense net-like meshwork. Modified from Mach et al. 2002. Neuroscience.

Fig. 2.

Representative radiographs showing a naïve, pin and pin+fracture femur in the female and male adult Sprague–Dawley rat. A stainless steel pin was implanted into the intramedullary space of the femur 21 days prior to fracture in order to provide mechanical stability to allow bone healing. Closed mid-diaphyseal fractures of the left femur were produced in female and male rats using a 3-point impactor device. Radiographic images of femurs from naïve (A, B), pin (C, D), and pin+fracture 2 days post-fracture (E, F). *Anesthesiology*;108(3):473–83, with permission.

Fig. 3.

Pain-related behaviors following a closed fracture of the femur in female and male rats. Female and male pin+fracture rats (closed squares) exhibited a greater time spent spontaneously guarding (A, B), a greater number of spontaneous flinches (C, D), and reduced weight bearing of the fractured limb (E, F) as compared to pin rats (open triangles) or age-matched naïve rats (closed circles). There were no differences in pain-related behaviors between female and male rats in nearly all time points. Data are presented as the

mean $\pm$ standard error of the mean. (* $p > 0.05$, Bonferroni-adjusted, vs. pin).
Anesthesiology;108 (3):473–83, with permission.

Fig. 4.

Soft callus formation, which results in stabilization of the fracture site, is shown at day 14 post-fracture by radiographic, micro-computed tomography, and histological analysis. At day 14 post-fracture, calcification of the callus around the fracture site has begun in female and male rats as shown in the radiographs (A, B) and 3D micro-computed tomography images (C, D) of the mid-diaphysis. Additional soft callus formation has occurred at day 14 post-fracture (hematoxylin and eosin) (E, F). This soft callus provides mechanical stabilization of the fractured bone and may in part be responsible for attenuation of acute fracture pain. Scale bar=3.0 mm. *Anesthesiology*;108(3):473–83, with permission.

Fig. 5.

Fracture-induced skeletal pain-related behaviors are significantly attenuated by anti-NGF therapy. Repeated administration of anti-NGF therapy (10 mg/kg; i.p. administered at day 1 and 6 post-fracture) significantly reduced fracture-induced spontaneous (A) and palpation-evoked (B) guarding behavior and number of spontaneous (C) and palpation-evoked flinches (D) at days 2, 8, and 12 post-fracture. Note that the anti-NGF anti-nociceptive effect was comparable to or greater than repeated injections (administered 15 min prior to evaluation at day 2, 8, and 12 post-fracture) of 10 mg/kg morphine sulfate (MS). Jimenez-Andrade et al. 2007. Pain. With permission. * $p < 0.005$ vs. fracture+vehicle. # $p < 0.05$ vs. fracture+MS.

Table 1

Comparison of evoked pain behaviors in models of postoperative pain

Model	Species	Duration of evoked mechanical hypersensitivity	Primary and secondary hyperalgesias?	Heat hyperalgesia	Cold allodynia	Reference
Incision in hindpaw skin/fascia/muscle	Rat	3–5 days	Both present	Present	Not reported	Brennan et al., 1996
Incision in back hairy skin	Rat	3–7 days	Both present	Not reported	Not reported	Duarte et al., 2005
Incision in hindlimb skin/fascia/muscle	Rat	8 days	Secondary only	Absent	Not reported	Pogatzki et al., 2002
Thoracotomy—skin/muscle incisions with rib retraction	Rat	At least 40 days	Primary reported	Not reported	Present	Buvanendran et al., 2004
Skin/muscle incision and retraction (SMIR) in medial thigh	Rat	22 days	Secondary only	Absent	Absent	Flatters, 2008