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Mouse Model of Fracture Pain

Vincent Minville, M.D., Jean-Michel Laffosse, M.D.,† Olivier Fourcade, M.D., Ph.D.,‡ Jean-Pierre Girolami, Ph.D.,§ Ivan Tack, M.D., Ph.D.‖*

Background: **The aim of this study was to validate a model of postfracture pain in mice, which was evaluated in the presence and the absence of morphine and ketoprofen.**

Methods: **The study was divided into two parts: protocol A, the effects of closed fracture; and protocol B, the effects of morphine and ketoprofen on fracture pain. In protocol A, mice were assigned to three groups: group 1, sham incision; group 2, sham pinning; or group 3, fracture. In protocol B, mice were randomly assigned to four groups to receive morphine (3 or 10 mg/kg body weight), ketoprofen (50 mg/kg body weight), or placebo (vehicle). Three tests were used to assess pain behavior: von Frey filament application, hot plate test, and a subjective pain scale.**

Results: **In protocol A, thermal nociception, mechanical nociception, and subjective pain were significantly modified in group 3 (fractured) compared with control groups 1 and 2 (sham groups). In protocol B, when tests were repeated for 240 min in morphine-treated animals and in ketoprofen-treated animals, reduction of mechanical nociception, thermal nociception, and subjective pain scale score were observed. Morphine and ketoprofen administration provided the same effect on behavioral testing on postoperative days 1 and 2.**

Conclusion: **This mouse model seems to be a reliable and reproducible tool to investigate the effect of closed bone fracture on several parameters, such as pain, remodeling, and recovery. Moreover, it allows studying the effects of various pharmacologic treatments as well as the involvement of various systems using different genetically modified strains of mice.**

POSTOPERATIVE pain management is a great challenge because it is a critical part of patient recovery.[1,2] Several animal pain models exist to assess the efficacy of drugs or to study the physiopathology of pain. These models include incisional pain,[3] neuropathic pain,[4,5] and inflammatory pain.[6–8] To our knowledge, no mouse model of posttraumatic fractured bone pain has been described in the literature. Although some fractured rodent models have been reported in mice,[9–12] they were mainly designed to assess bone reconstruction and not pain, which was evaluated only by simple subjective pain tests.[13,14]

Presumably, if we learn more about the etiology of acute bone fracture pain and the sensory processes that intensify pain after trauma, new treatment methods can be advanced. These models will improve our understanding of pain mechanisms caused by particular injuries. Understanding postoperative pain mechanisms will improve treatments and perioperative morbidity. Because of the increasing number of genetically modified strains of mice, the availability of a pain model will open new possibilities to investigate the systems involved in the physiopathologic mechanism.

Therefore, the aim of this study was to validate a model of postfracture pain in mice, which was evaluated in the presence and the absence of morphine or ketoprofen.

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Materials and Methods

Animals

This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (I.T.). These experiments were approved by our institutional animal care and use committee, and this study was conducted in accordance with the International Association for the Study of Pain Guidelines on the Use of Animals in Experimental Research.[15] Adult C57 BL/6 male mice (The Jackson Laboratories, Bar Harbor, ME) were used in all experiments. The animals were housed individually in isolator cages with solid floor covered with 3 cm of soft bedding and were fed and watered *ad libitum*. Animals were on a 12-h light–dark cycle.

Surgery

All mice were anesthetized with 1.5–2% sevoflurane delivered *via* cone nose. After antiseptic preparation of the right paw with povidone iodine, a unilateral, closed fracture was produced in the right tibia using a specially designed fracture apparatus (blunt guillotine). The fracture apparatus consists of four parts: a frame, an animal support system, a guillotine ramming system, and a 300-g weight. The support anvil was made with an adjustable

* Staff Anesthesiologist, Department of Anesthesiology and Intensive Care, GRCB 48, Toulouse University Hospital; Physiology Laboratory, Institut National de la Santé et de la Recherche Médicale (INSERM) U 858, Toulouse University Hospital Rangueil. † Staff Orthopedist, Department of Orthopedic and Traumatology Surgery, Toulouse University Hospital Rangueil. ‡ Professor of Anesthesiology, Department of Anesthesiology and Intensive Care, GRCB 48, Toulouse University Hospital. § Director of Research, ‖ Professor of Physiology, Physiology Laboratory, INSERM U 858, Toulouse University Hospital Rangueil.

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Address correspondence to Dr. Minville: Department of Anesthesiology and Intensive Care, Rangueil University Hospital, Avenue Jean Poulhès, Toulouse, France. minville.v@chu-toulouse.fr.

foot rest that ensures that all of the fractures are at the same level by positioning the mouse leg on the anvil with the foot against the foot rest. For the intramedullary pinning using a sterile technique, a hole was made above the tibial tuberosity percutaneously using a 27-gauge needle (Becton Dickinson and Company, Drogheda, Ireland). Then the needle was directed directly into the medullary canal. By rotating the needle, the canal was reamed to 5 mm up to the ankle joint. The end of the needle was cut as short as possible so that the skin could roll over and cover it. No suture was used.

Then, the mouse was placed with the leg on the anvil so that the blunt guillotine lined up with the proximal third of the tibia. The 300-g weight was dropped from a height of 9–10 cm, fracturing the tibia shaft. Other heights were tested, but this one produced the best results. Radiography confirmed the fracture.

Experimental Groups

Protocol A: Effects of Closed Fracture. In group 1 (sham incision group, n = 10) mice were anesthetized, and a skin puncture of the knee was performed using the same 27-gauge needle.

In group 2 (sham pinning group, n = 10) mice were anesthetized, and an intramedullary pinning was placed as described above, but no fracture was performed.

In group 3 (fracture group, n = 10) mice were anesthetized, an intramedullary pinning was placed, and a closed fracture was performed as described above.

All mice of each group underwent postoperative pain testing. The animals were tested before surgery (baseline); 2, 4, and 6 h after surgery; once daily during the first 7 postoperative days; and the week after surgery.

Protocol B: Effects of Morphine and Ketoprofen on Fracture Pain. The aim of this second part was to assess the effect of systemic morphine administration on enhanced responses to postoperative testing.

Forty other mice were randomly assigned to receive morphine (3 or 10 mg/kg body weight), ketoprofen (50 mg/kg body weight), or placebo (vehicle). Surgery was performed as described above. Testing (mechanical stimulation, hot plate test, and pain rating scale) were performed before the surgery and 2 h after the surgery (T0 = baseline). Then mice were randomly assigned to receive 3 mg/kg morphine (n = 10), 10 mg/kg morphine (n = 10), ketoprofen (n = 10), or a saline vehicle subcutaneously (n = 10). Responses to mechanical and heat stimuli and pain rating scale score were determined each 30 min after T0 until 240 min after the administration. The effects of morphine administration and of ketoprofen administration were also assessed on behavioral testing on postoperative days 1 and 2 using the same procedure. Experiments were conducted following a double-blind protocol.

Behavioral Measurements

Three tests were used to assess pain behavior: (1) mechanical nociception assessed by the withdrawal response to von Frey filament application, (2) thermal nociception assessed by the withdrawal response to thermal stimulus (hot plate test), and (3) subjective pain determined using a pain rating scale as described by Attal *et al.*[16]

Mechanical Nociception. Unrestrained mice were placed beneath a clear plastic chamber on an elevated mesh floor and allowed to acclimate. Withdrawal responses to mechanical stimulation were determined using calibrated von Frey filaments applied from underneath the cage through openings in the plastic mesh floor against the hind paw plantar skin at approximately the middle of the paw at the fractured side. The filament was pushed until it slightly bowed and then it was jiggled in that position for 6 s. Each von Frey filament was applied once, starting with 0.008 g and continuing until a withdrawal response was reached that was considered a positive response. After a 5- to 10-min rest period, each filament was again applied once, beginning with 0.008 g until a withdrawal response was elicited. This was repeated a third time 5–10 min later. The lowest force from the three tests producing a response was considered the withdrawal threshold.

Thermal Nociception. Thermal nociception was measured by a modified hot plate test.[17] The time that a mouse would leave its hind paw on a hot plate at 52°C reflects thermal nociception (thermal latency). The paw was removed from the plate after a maximal time of 12 s by the investigator to avoid thermal injury and thermal hyperalgesia.[17] This test was repeated three times on each hind paw for each mouse.

Subjective Pain Scale. A subjective pain rating scale (0–5) modified from that described by Attal *et al.*[16] was used to quantify the pain, where 0 is normal, 1 is curling of the toes, 2 is eversion of the paw, 3 is partial weight bearing, 4 is non–weight bearing and guarding, and 5 is avoidance of any contact with the hind limb.

Statistical Analysis

The results of behavioral testing were not normally distributed and thus were analyzed nonparametrically. To assess whether the withdrawal responses changed over time, the Friedman test was used. When the Friedman test was significant ($P < 0.05$), pairwise comparisons were made using the Wilcoxon signed rank test. Time point comparisons between groups were made using first a nonparametric Kruskal-Wallis test. When the Kruskal-Wallis test was significant ($P < 0.05$), pairwise comparisons were made using the Mann–Whitney U test. The effect sizes were also estimated using Cohen d values. Cohen d values are considered to be a small effect size at 0.2, a moderate effect size at 0.5, and a large effect size at greater than 0.8.

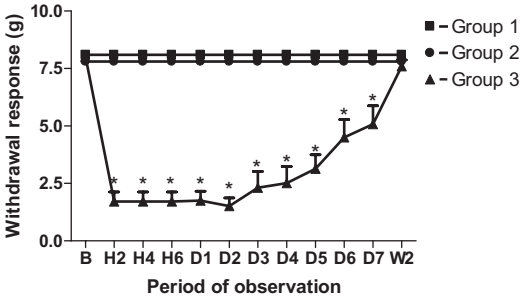

Fig. 1. Withdrawal response (grams) to von Frey filaments assessing mechanical hyperalgesia in the fractured hind paw. Group 1 mice were anesthetized, and a skin puncture of the knee was performed using the same 27-gauge needle; group 2 mice were anesthetized, and an intramedullary pinning was placed as described above, but no fracture was performed; group 3 mice were anesthetized, an intramedullary pinning was placed, and a closed fracture was performed as described in the Materials and Methods. *Symbols* represent median ± interquartile range. * $P < 0.05$ *versus* groups 1 and 2.

Results

Throughout the experimental period, all mice remained well groomed and maintained normal food and water intake. No signs of spontaneous pain behavior, such as licking, biting, or flinching, were noticed after the surgery. The experiments were conducted following a double-blind procedure because neither swelling nor hematoma occurred after fracture.

Protocol A: Effects of Closed Fracture

No difference in the measured parameter was observed in any of the groups at baseline.

Thermal nociception, mechanical nociception, and subjective pain were significantly modified in group 3 compared with control groups 1 and 2.

As shown in figure 1, withdrawal response to mechanical stimulus decreased in group 3, indicating an increased nociception, whereas no change was observed in groups 1 and 2.

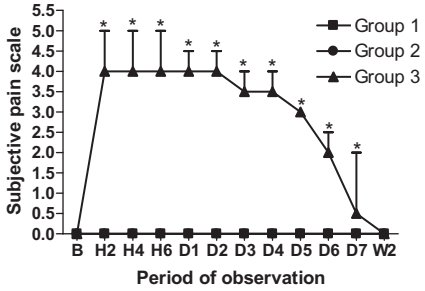

Fig. 2. Thermal nociceptive withdrawal latency (seconds) assessing thermal hyperalgesia in the fractured hind paw. Group 1 mice were anesthetized, and a skin puncture of the knee was performed using the same 27-gauge needle; group 2 mice were anesthetized, and an intramedullary pinning was placed as described above, but no fracture was performed; group 3 mice were anesthetized, an intramedullary pinning was placed, and a closed fracture was performed as described in the Materials and Methods. *Symbols* represent median ± interquartile range. * $P < 0.05$ *versus* groups 1 and 2.

Table 1. Effect Sizes (Cohen d) after Mechanical Stimulation in Protocol A

Time of Observation	Fracture Compared with Sham Pinning	Fracture Compared with Sham Incision
Hour 2	6.75	6.75
Hour 4	6.75	6.75
Hour 6	6.75	6.75
Day 1	6.80	6.80
Day 2	8.09	8.09
Day 3	3.60	3.60
Day 4	3.39	3.39
Day 5	3.57	3.57
Day 6	2.01	2.01
Day 7	0.88	0.88
Week 2	0.79	0.79

Effect sizes (Cohen d) for fracture compared with sham pinning and incision regarding the response to mechanical stimulation (von Frey filaments) in protocol A. Cohen d <0.2, small effect; 0.5, moderate effect; and >0.8, large effect.

The same pattern was observed in figure 2; the response latency reflecting an increased nociception was significantly decreased in group 3, whereas no change was observed in groups 1 and 2. Effect sizes (Cohen d) for mechanical and thermal stimulation are shown in tables 1 and 2, respectively.

The subjective pain scale shown in figure 3 was significantly increased in group 3 compared with groups 1 and 2, which remained steady.

Protocol B: Effects of Morphine and Ketoprofen on Fracture Pain

When tests were repeated for 240 min in morphine- or ketoprofen-treated animals, reduction of mechanical nociception, thermal nociception, and subjective pain scale score were observed.

As shown in figures 4 and 5, withdrawal response and thermal response increased transiently after a single morphine injection. Moreover, the increase was dose dependent. As shown in figures 4 and 5, withdrawal response

Table 2. Effect Sizes (Cohen d) after Thermal Stimulation in Protocol A

Time of Observation	Fracture Compared with Sham Pinning	Fracture Compared with Sham Incision
Hour 2	6.73	6.73
Hour 4	12.13	12.13
Hour 6	7.80	7.80
Day 1	3.73	3.73
Day 2	3.23	3.23
Day 3	2.77	2.77
Day 4	2.14	2.14
Day 5	2.61	2.61
Day 6	1.84	1.84
Day 7	1.78	1.78
Week 2	0.45	0.45

Effect sizes (Cohen d) for fracture compared with sham pinning and incision regarding the response to thermal stimulation (hot plate test) in protocol A. Cohen d <0.2, small effect; 0.5, moderate effect; and >0.8, large effect.

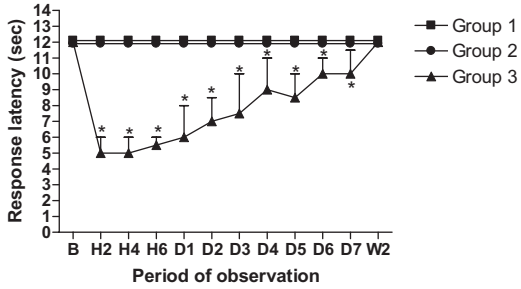

Fig. 3. Subjective pain scale assessing pain in the fractured hind paw. Group 1 mice were anesthetized, and a skin puncture of the knee was performed using the same 27-gauge needle; group 2 mice were anesthetized, and an intramedullary pinning was placed as described above, but no fracture was performed; group 3 mice were anesthetized, an intramedullary pinning was placed, and a closed fracture was performed as described in the Materials and Methods. *Symbols* represent median ± interquartile range. * $P < 0.05$ *versus* groups 1 and 2.

and thermal response increased transiently after a single ketoprofen injection. Effect sizes (Cohen d) for mechanical and thermal stimulation are shown in tables 3 and 4, respectively. The subjective pain scale (fig. 6) was transiently reduced in a dose-dependent manner after morphine injection and returned to maximal value after 240 min. It was also transiently reduced after ketoprofen injection (fig. 6). The effects of morphine administration and of ketoprofen administration were identical on behavioral testing on postoperative days 1 and 2, as shown in insets of figures 4–6.

Discussion

The current study developed a method of closed fracture of the tibia shaft to validate a model of postfracture bone pain in mice. Because no change was observed in the control groups (groups 1 and 2), it can be reasonably assumed that the effects observed in the fractured group (group 3) result from events occurring at the site of the bone lesion. Moreover, this model is morphine– and

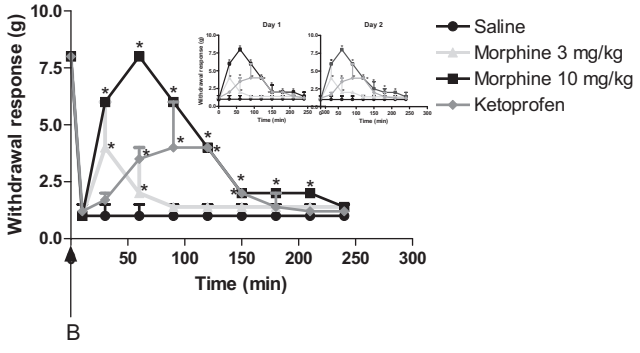

Fig. 4. Effect of subcutaneous morphine and ketoprofen on withdrawal response (grams) to von Frey filaments assessing mechanical hyperalgesia in the fractured hind paw. *Symbols* represent median ± interquartile range. * $P < 0.05$ *versus* saline group.

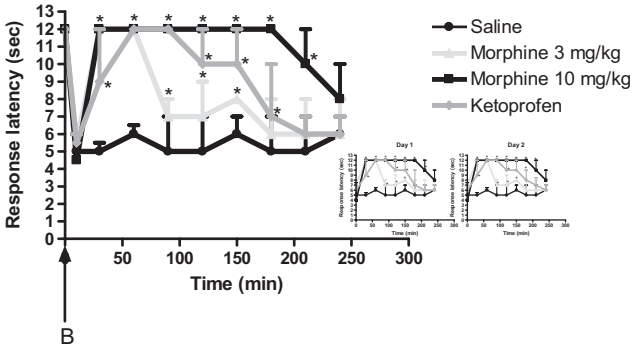

Fig. 5. Effect of subcutaneous morphine and ketoprofen on thermal nociceptive withdrawal latency (seconds) assessing thermal hyperalgesia in the fractured hind paw. *Symbols* represent median ± interquartile range. * $P < 0.05$ *versus* saline group.

nonsteroidal antiinflammatory drug (NSAID)–sensitive, a situation closely related to clinical practice.

To our knowledge, this is the first report of posttraumatic model in mice. However, one model after bone injury has been recently reported in rats.[18] Besides the considerable advantage of using a mouse model *versus* a rat model (genetically modified mice), the described rat model was different. In this model, bone lesions were induced by bone holing after skin incision, thereby resulting in several origins of pain mechanism (bone, skin, muscles, and so on).

The mouse model of standard closed tibia fracture described here has been adapted from a rat model of closed tibia fracture.[10,12] However, this model was only used by orthopedists to assess bone reconstruction.[13,14] In a recent model described by Bonnarens *et al.*, closed femur fracture was used in mice,[9,11] mainly for evaluation of bone reconstruction. They used only a simple subjective pain evaluation.[19,20] However, in our model, the tibia (and not the femur) was fractured, producing a pain mainly due to bone fracture (and probably less to hematoma and inflammation) and allowing us more easily, because of its peripheral localization in the limb, to accurately evaluate pain, *i.e.*, use of mechanical and thermal stimulation.

Table 3. Effect Sizes (Cohen d) after Mechanical Stimulation in Protocol B

Time, min	Ketoprofen	3 mg/kg Morphine	10 mg/kg Morphine
30	0.82	2.07	2.60
60	1.90	1.48	11.40
90	1.95	0.86	12.90
120	2.84	0.86	2.93
150	2.34	0.86	1.85
180	0.82	0.52	2.34
210	0.20	0.52	2.34
240	0.10	0.52	0.52

Effect sizes (Cohen d) for ketoprofen, 3 mg/kg morphine, and 10 mg/kg morphine compared with placebo (saline) regarding the response to mechanical stimulation (von Frey filaments) in protocol B. Cohen d <0.2, small effect; 0.5, moderate effect; and >0.8, large effect.

Table 4. Effect Sizes (Cohen d) after Thermal Stimulation in Protocol B

Time, min	Ketoprofen	3 mg/kg Morphine	10 mg/kg Morphine
30	2.05	11.13	14.89
60	7.03	8.42	8.42
90	5.91	1.27	2.24
120	4.00	1.60	2.61
150	2.52	1.70	2.43
180	1.33	0.92	2.10
210	0.73	0.44	1.84
240	0.53	0	1.25

Effect sizes (Cohen d) for ketoprofen, 3 mg/kg morphine, and 10 mg/kg morphine compared with placebo (saline) regarding the response to thermal stimulation (hot plate test) in protocol B. Cohen d <0.2, small effect; 0.5, moderate effect; and >0.8, large effect.

In clinical practice, we know that pain due to a fractured limb could be a challenge because it could be an early step in chronic pain and complex regional pain syndrome type I.[21–23] We decided to adapt this model from rats to mice because of the accessibility of genetically modified mice. A similar modification of a rat model has been reported by Pogatzki and Raja[3] to study mechanisms involved in postincisional pain in mice. This approach further allowed these authors to investigate neurobiologic mechanism of pain after surgery, pointing out the interest of using genetically modified mice.[24,25]

Our model will probably be useful to study the different pain mechanisms involved in postfracture pain. It will also be valuable to study a variety of pharmacologic treatments. Indeed, as shown in the current study, the pain after bone trauma is sensitive to morphine and NSAIDs. Presumably, peripheral opioid receptors are involved in pain, but the precise mechanism has not been documented yet. It has been reported that opioids resulted in clear analgesic as well as remodeling properties (apoptosis, cell proliferation and growth) in animal models of cancer pain.[26] Because of the lack of a postfracture pain model, this effect remains unknown in bone trauma and needs to be investigated. The antiinflammatory and antinociceptive activities of NSAIDs are attributed to the inhibition of cyclooxygenase enzymes, decreasing in turn the synthesis of prostaglandins that promote inflammatory responses and enhanced sensitivity to pain at the peripheral site of tissue injury.[27] It has been shown that NSAIDs impaired fracture healing.[28,29] Interestingly, the current study indicates that drugs activating opioid receptors and affecting the cyclooxygenase pathway reduce pain behavior in this model. Whether other different systems are also involved will be investigated using different genetically modified strains of mice. Moreover, in these future studies, the current model will probably allow discrimination between the analgesic effect and the remodeling effect of a given drug. However, the limitations of animal models of bone pain analgesics like NSAIDs may impair healing, but with pain relief, weight bearing and increased activity may impair fracture healing as well. Any sustained analgesic effect may affect activity and weight bearing, and this may contribute to changes in remodeling, healing, and so on.

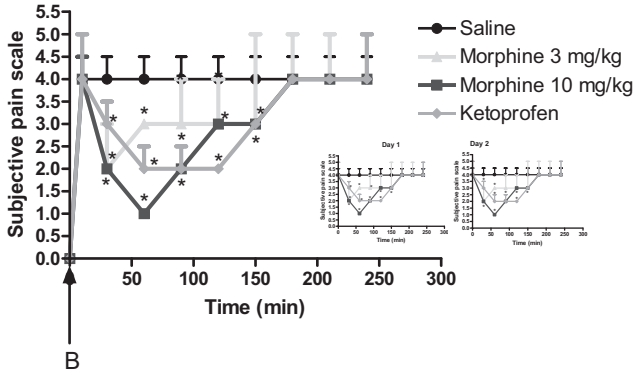

Fig. 6. Effect of subcutaneous morphine and ketoprofen on subjective pain scale. *Symbols* represent median ± interquartile range. * $P < 0.05$ *versus* saline group.

In summary, our model seems to be a reliable and reproducible tool to investigate the effect of closed bone fracture on several parameters, such as pain, remodeling, and recovery. Moreover, it allows studying the effects of various pharmacologic treatments.

References

1. Kehlet H, Dahl JB: Anaesthesia, surgery, and challenges in postoperative recovery. Lancet 2003; 362:1921–8
2. Kehlet H, Jensen TS, Woolf CJ: Persistent postsurgical pain: risk factors and prevention. Lancet 2006; 367:1618–25
3. Pogatzki EM, Raja SN: A mouse model of incisional pain. ANESTHESIOLOGY 2003; 99:1023–7
4. Hosl K, Reinold H, Harvey RJ, Muller U, Narumiya S, Zeilhofer HU: Spinal prostaglandin E receptors of the EP2 subtype and the glycine receptor alpha3 subunit, which mediate central inflammatory hyperalgesia, do not contribute to pain after peripheral nerve injury or formalin injection. Pain 2006; 126:46–53
5. Bennett GJ, Xie YK: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33:87–107
6. Beloeil H, Asehnoune K, Moine P, Benhamou D, Mazoit JX: Bupivacaine's action on the carrageenan-induced inflammatory response in mice: Cytokine production by leukocytes after ex-vivo stimulation. Anesth Analg 2005; 100: 1081–6
7. Nagae M, Hiraga T, Wakabayashi H, Wang L, Iwata K, Yoneda T: Osteoclasts play a part in pain due to the inflammation adjacent to bone. Bone 2006; 39:1107–15
8. Beloeil H, Ji RR, Berde CB: Effects of bupivacaine and tetrodotoxin on carrageenan-induced hind paw inflammation in rats: II. Cytokines and p38 mitogen-activated protein kinases in dorsal root ganglia and spinal cord. ANESTHESIOLOGY 2006; 105:139–45
9. Bonnarens F, Einhorn TA: Production of a standard closed fracture in laboratory animal bone. J Orthop Res 1984; 2:97–101
10. Bak B, Jensen KS: Standardization of tibial fractures in the rat. Bone 1992; 13:289–95
11. Manigrasso MB, O'Connor JP: Characterization of a closed femur fracture model in mice. J Orthop Trauma 2004; 18:687–95
12. An Y, Friedman RJ, Parent T, Draughn RA: Production of a standard closed fracture in the rat tibia. J Orthop Trauma 1994; 8:111–5
13. El-Zawawy HB, Gill CS, Wright RW, Sandell LJ: Smoking delays chondrogenesis in a mouse model of closed tibial fracture healing. J Orthop Res 2006; 24:2150–8
14. Lu C, Miclau T, Hu D, Marcucio RS: Ischemia leads to delayed union during fracture healing: A mouse model. J Orthop Res 2007; 25:51–61
15. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109–10
16. Attal N, Jazat F, Kayser V, Guilbaud G: Further evidence for "pain-related" behaviours in a model of unilateral peripheral mononeuropathy. Pain 1990; 41:235–51

17. Lee KC, Wilder RT, Smith RL, Berde CB: Thermal hyperalgesia accelerates and MK-801 prevents the development of tachyphylaxis to rat sciatic nerve blockade. ANESTHESIOLOGY 1994; 81:1284–93

18. Houghton AK, Hewitt E, Westlund KN: Enhanced withdrawal responses to mechanical and thermal stimuli after bone injury. Pain 1997; 73:325–37

19. Koewler NJ, Freeman KT, Buus RJ, Herrera MB, Jimenez-Andrade JM, Ghilardi JR, Peters CM, Sullivan LJ, Kuskowski MA, Lewis JL, Mantyh PW: Effects of a monoclonal antibody raised against nerve growth factor on skeletal pain and bone healing after fracture of the C57BL/6J mouse femur. J Bone Miner Res 2007; 22:1732–42

20. Jimenez-Andrade JM, Martin CD, Koewler NJ, Freeman KT, Sullivan LJ, Halvorson KG, Barthold CM, Peters CM, Buus RJ, Ghilardi JR, Lewis JL, Kuskowski MA, Mantyh PW: Nerve growth factor sequestering therapy attenuates non-malignant skeletal pain following fracture. Pain 2007; 133:183–96

21. Smith EJ, Ward AJ, Watt I: Post-traumatic osteoporosis and algodystrophy after external fixation of tibial fractures. Injury 1993; 24:411–5

22. Thomas B, Yeo JM, Slater GL: Chronic pain after ankle fracture: An arthroscopic assessment case series. Foot Ankle Int 2005; 26:1012–6

23. Sarangi PP, Ward AJ, Smith EJ, Staddon GE, Atkins RM: Algodystrophy and osteoporosis after tibial fractures. J Bone Joint Surg Br 1993; 75:450–2

24. Zahn PK, Pogatzki EM, Brennan TJ: Mechanisms for pain caused by incisions. Reg Anesth Pain Med 2002; 27:514–6

25. Brennan TJ, Zahn PK, Pogatzki-Zahn EM: Mechanisms of incisional pain. Anesthesiol Clin North Am 2005; 23:1–20

26. El Mouedden M, Meert TF: The impact of the opioids fentanyl and morphine on nociception and bone destruction in a murine model of bone cancer pain. Pharmacol Biochem Behav 2007; 87:30–40

27. Dogrul A, Gulmez SE, Deveci MS, Gul H, Ossipov MH, Porreca F, Tulunay FC: The local antinociceptive actions of nonsteroidal antiinflammatory drugs in the mouse radiant heat tail-flick test. Anesth Analg 2007; 104:927–35

28. Bergenstock M, Min W, Simon AM, Sabatino C, O'Connor JP: A comparison between the effects of acetaminophen and celecoxib on bone fracture healing in rats. J Orthop Trauma 2005; 19:717–23

29. Murnaghan M, Li G, Marsh DR: Nonsteroidal anti-inflammatory drug-induced fracture nonunion: An inhibition of angiogenesis? J Bone Joint Surg Am 2006; 88 (suppl 3):140–7