Clin Orthop Relat Res (2013) 471:4076–4081
DOI 10.1007/s11999-013-3232-z

Clinical Orthopaedics and Related Research®
A Publication of The Association of Bone and Joint Surgeons®

BASIC RESEARCH

Postoperative Opioid Administration Inhibits Bone Healing in an Animal Model

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Received: 14 March 2013 / Accepted: 5 August 2013 / Published online: 17 August 2013

Abstract

Background The current mainstay of orthopaedic pain control is opioid analgesics but there are few studies in the literature evaluating the effects of opioids on bone healing.

Questions/purposes The purpose of this study was to use a rat fracture model to evaluate the effects of opioid administration on osseous union in the acute (4 weeks) and subacute (8 weeks) setting in an operatively stabilized fracture. We asked the following question: does morphine administration alter (1) fracture callus strength; (2) callus volume and formation; and (3) morphology and early remodeling to final osseous union?

Methods A 0.4-mm femoral osteotomy gap was created in 50 Sprague-Dawley rats using an established model. Postoperatively, rats were randomized to control versus morphine-treated study groups. Equal numbers of rats from each group were euthanized at 4 weeks and 8 weeks postoperatively. Three-point bend biomechanical testing was performed to evaluate postoperative callus strength. Micro-CT scans and histological analyses were used to evaluate postoperative callus volume and formation, morphology, and features of early remodeling.

Results Biomechanical testing identified a statistically significant ($p = 0.048$) reduction in callus strength in morphine-treated animals 8 weeks postoperatively compared with controls. Radiographic and histological analysis showed delayed callus maturation and lack of remodeling in the morphine group compared with control animals at 8 weeks. Micro-CT analysis expressed remodeling and resorption as a decrease in callus volume over the two time points. The control group had significant levels of resorption decreasing 29% ($p = 0.023$) over the 4-week to 8-week time interval. Morphine administration inhibited callus resorption and remodeling with only a 13% decrease ($p = 0.393$) in callus volume comparing these time points. The callus inhibition associated with morphine administration was not as evident in the acute, 4-week time setting.

Conclusions Morphine administration inhibited callus strength in this animal model. This finding is likely consistent with the observation that the callus and healing bone appear to have a decreased rate of maturation and remodeling seen at 8 weeks.

Clinical Relevance This study identifies that administration of an opioid pain medication leads to weaker callus and impedes callus maturation compared with controls.

Funding for this study was provided by an Orthopaedic Research and Education Foundation's (OREF)/Synthes Resident Research Project Grant and a departmental grant from the Sherman S. Coleman Resident Research Fund. Synthes Research Project Support donated surgical supplies/instruments. One of the authors (KBJ) receives career development support from the National Cancer Institute (K08CA138764).

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request.

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Each author certifies that his or her institution approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

All work was performed at the University of Utah campus, Salt Lake City, UT, USA.

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These findings may provide the impetus to alter our current orthopaedic analgesic gold standard toward more multimodal and opioid-limiting pain control regimens.

Introduction

Osseous union and bone remodeling to achieve premorbid strength remain important objectives of orthopaedic fracture care. Several modifiable risk factors are known to affect the healing of osteotomies and fractures. Smoking [6, 12], diabetes [8], obesity [6], and endocrinopathies [3] such as hypogonadism, vitamin D deficiencies, and calcium imbalances have all been identified as patient-specific risk factors for delayed union and nonunion. Iatrogenic concerns for impaired healing have also been linked to medications such as nonsteroidal antiinflammatory drugs [2, 8, 13]; this research has resulted in decreased use of these drugs in patients with fracture.

Opioid analgesics currently are the mainstay of postoperative pain control. However, a study by Bhattacharyya et al. [2] raised concern for opioid pain medications contributing to nonunion in humeral shaft fractures. Beyond that report, no other clinical study has been performed looking at this relationship. Opioid use in medicine and in particular the orthopaedic population continues to rise dramatically in the United States. Greater than 80% of orthopaedic patients are prescribed some form of opioid analgesic in the perioperative or fracture care period [1, 10]. If these drugs indeed affect fracture healing, that would be critically important to know; despite continued advances in implants and technology, approximately 5% to 10% of all patients have problems obtaining final union of their fractures [5, 14]. If a correlation were drawn between impaired bone healing and the use of opioid analgesics, we would need to reconsider our analgesic approaches, including the intensity and duration of narcotic pain medication use in operative and nonoperative fracture care.

The purpose of this study was to use a rat fracture model to evaluate the effects of opioid administration on osseous union in the acute (4 weeks) and subacute (8 weeks) setting in an operatively stabilized fracture. We asked the following question: does morphine administration alter (1) fracture callus strength; (2) callus volume and formation; and (3) morphology and early remodeling to final osseous union?

Materials and Methods

Study Design

A rat femur diaphyseal fracture model described by Schmidhammer et al. [15] was used. Fifty adult male Sprague-Dawley rats (Harlan® Laboratories, Indianapolis, IN, USA) weighing 300 g each were used in this study. Institutional Animal Care and Use Committee approval was obtained before study initiation (11-05012). Animals were quarantined for 1 week as per the institution standard. Subjects were housed in groups of two to three per cage with standard 12-hour light/dark cycles observed and ad libitum food and water consumption.

A single surgeon conducted all surgeries. Inhaled isoflurane anesthesia was administered per standard rat anesthesia protocol with an induction rate of 3% to 5% and a maintenance rate of 1% to 3%. The subjects were anesthetized for the entirety of the procedure. The animal's right thigh and hip were shaved and cleansed. A lateral approach to the femur was performed; care was taken to minimize disruption of the periosteum. The osteotomy was performed and stabilized with a nonlocking 1.5-mm plate and four screws (Synthes, West Chester, PA, USA). The diaphyseal osteotomy was performed between the center two holes using a Stryker TPS system and Stryker Precision™ Thin blade (Stryker, Kalamazoo, MI, USA) leaving the kerf cut of the blade (0.38 mm) as our osteotomy gap. The wound was closed in layers with 3-0 Monocryl and the skin closed with staples. The plate-stabilized osteotomy model was used for this study to limit potential variables. Closed fracture models can lead to varying degrees of comminution and soft tissue injury and can be more difficult to standardize. The plate fixation was used to stabilize the osteotomy kerf gap in hopes of better evaluating the bridging callus volume and formation on histology and advanced imaging. After the procedure, the animals were transferred to a recovery area where they were monitored and allowed to awaken from anesthesia. Animal randomization occurred postoperatively with the animals receiving their randomized medication within the first 5 minutes of awakening from anesthesia.

The 50 animals were assigned equally to either saline (control) or opioid administration (morphine) study arms. All animals received two doses of postoperative acetaminophen (300 mg/kg) administered 12 hours apart by oral gavage. The control group received 0.9% subcutaneous saline injections every 8 hours (100 μL/kg) throughout the duration of the experiment. The morphine group received subcutaneous morphine injections (Hospira Inc, Lake Forest, IL, USA) (5 mg/100 μL/kg) every 8 hours throughout the duration of the experiment. The animals were all returned to their cages and allowed to mobilize ad libitum. Animals were weighed on a weekly basis and medications adjusted to compensate for alterations in weight.

At the 4-week time point, half of the animals from each group were randomly selected and euthanized. At 8 weeks postoperatively, the remaining animals were euthanized.

Immediately postmortem, the bilateral femurs were disarticulated and dissected. The femora were stripped of muscle with care taken not to disrupt periosteal callus formation. The plate was removed from the operated femora. Micro-CT (μCT) was performed using a Siemens Inveon System μCT scanner (Siemens Preclinical, Knoxville, TN, USA) with a 35-μm voxel size.

There was one death among the control animals in the first 48 hours postoperatively. No other unintentional animal deaths occurred. There were no wound complications, infections, or injection site complications. The experimental animals demonstrated no differences in appetite or food consumption with no significant disparities in animal weight during the experiment. There were no noticeable signs of sedation or altered activity level in either the control or experimental animal groups throughout the course of the study.

After CT scanning, the femurs underwent three-point load to failure biomechanical testing using an Instron servo hydraulic testing device (Instron®, Norwood, MA, USA). The femurs were kept moist with 0.9% saline solution up to the time of testing. The femurs were placed on their posterior surface horizontally across two cylindrical supports spaced 25 mm apart with a force directed over the osteotomy site centered between the cylindrical supports to obtain measurements per Schmidhammer et al. [15]. The femur was compressed at a constant displacement rate of 0.1 mm/s until failure measuring load and displacement. Both the operative and nonoperative femurs underwent three-point load to failure biomechanical testing. A ratio of operative femur strength to nonoperative femur strength was obtained to calculate percent strength ratio to account for variability among the animals.

The μCT data were analyzed using AMIRA software (Visage Imaging® AMIRA 5.4.1; Visualization Sciences Group, Mérignac, France). One hundred sequential axial slices, centered around the osteotomy, were uploaded into the program. The callus was qualitatively evaluated for callus formation, maturation, and final union by two observers (JC, CS). The uploaded μCT data were then quantitatively analyzed. Any additional soft tissues and existing cortical bone were manually subtracted from the analysis. The callus volume was calculated for each osteotomized femora with surface area three-dimensional reconstruction and volumetric quantification using the AMIRA software.

Histologic studies were performed on the osteotomized femora. The femora were fixed in a buffered 10% formalin solution, decalcified, and embedded in a paraffin block. Serial cross-sections were taken including and adjacent to the osteotomy site. Sections were stained with hematoxylin and eosin (H&E) or trichrome stain. Two observers (JC, CS) qualitatively evaluated postmortem histologic sectioning of the fractured femurs in a blinded fashion. H&E stain and trichrome stain were used to evaluate callus maturity. The sections were evaluated at five times magnification to evaluate gross levels of healing with evidence of consolidation and remodeling as well as 100 times magnification to qualitatively assess fibrous tissue development, cartilage, woven immature bone and lamellar mature bone, callus consolidation and remodeling, and final osteotomy union of the osteotomized femora.

Statistical Analysis

Sample size was based on previous literature involving nonsteroidal antiinflammatory fracture healing in an animal model. The power analysis determined 19 animals would be needed per group to achieve a desired alpha level of 0.05 and an 80% power of detecting a 5% difference in fracture healing rates [4]. In this opioid study, we increased the sample size from 19 to 25 to account for potential perioperative or postoperative animal loss. Two-tailed Student's t-tests were conducted comparing the control group with the experimental morphine group. T-tests were conducted comparing callus strength ratio and callus volumes. Calculations were performed with STATA 12.1 (StataCorp LP, College Station, TX, USA).

Results

Morphine administration resulted in weaker fracture callus when measured 8 weeks after surgery. Comparing two study groups, there was a significant (control: 95% confidence interval [CI], 35–63; morphine: 95% CI, 17–43; $p = 0.048$) difference in final callus strength suggesting morphine decreases bony healing strength at 8 weeks (Fig. 1). At 8 weeks postoperatively, there was an expected and substantial increase in callus strength in the control arm with 49% of the contralateral femur. This was less dramatic in the experimental arm, rising only to 30% of the contralateral femur. At 4 weeks postoperatively, there was no difference comparing the control with morphine animals with regard to biomechanical strength (17% versus 16% of contralateral femur, respectively; $p = 0.868$).

Morphine decreased the rate of callus maturation and callus remodeling to final union at 8 weeks postoperatively on qualitative and quantitative assessment by μCT. Callus evaluation by μCT at 8 weeks postoperatively appeared to show more callus remodeling and osteotomy healing in the control group than seen in the experimental group. The morphine group had a persistence of immature callus with minimal filling of the osteotomy gap and persistence of fibrous interposition. These findings were not as apparent at

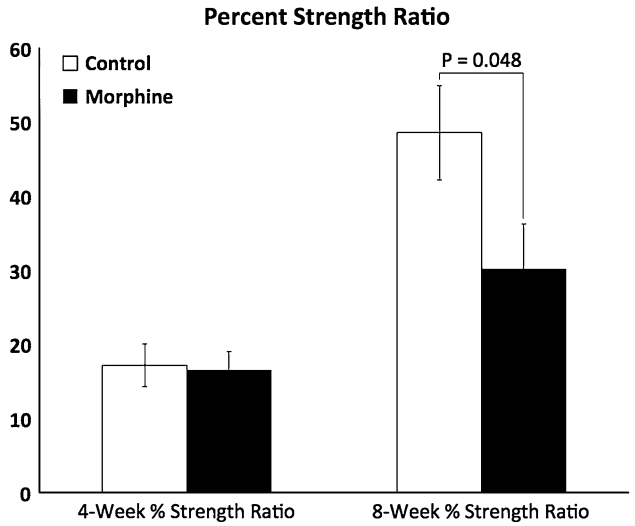

Fig. 1 Mean ratio ± SE of operative femur strength to nonoperative femur strength (measured as percent strength) at 4 weeks and 8 weeks from a three-point load to failure test comparing control animals with morphine-treated animals. No statistical difference was seen at 4 weeks. Morphine-treated animals were identified to have weaker callus strength compared with controls at 8 weeks postoperatively ($p = 0.048$).

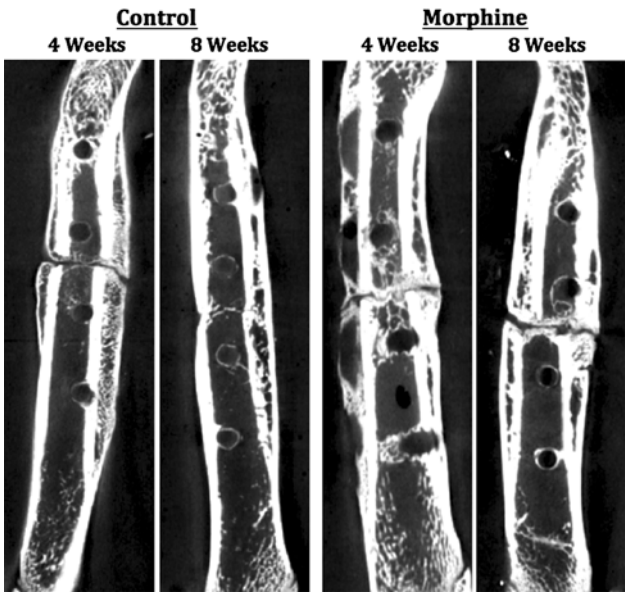

Fig. 2 Micro-CT images at 4 weeks and 8 weeks postoperatively. Gross evaluation of μCT images at 4 weeks reveals immature callus surrounding the osteotomy site, lack of bridging bone, and persistence of osteotomy lucency. The control and morphine groups have a qualitatively similar appearance at the 4-week time point. More notable differences can be seen at the 8-week time point. Callus resorption and interval cortical bridging are seen in the control group, whereas this is less apparent in the morphine group. There is less evidence of remodeling and persistent fibrous interposition at the osteotomy site.

the 4-week postoperative time point with similar levels of fibrous interposition, osteotomy healing, and callus maturation (Fig. 2). Quantitative analysis of the μCT data substantiated this decreased rate of callus maturation. There were no significant differences identified in the 4-week volumetric analysis for the total callus (49.7 mm^3 versus 51.7 mm^3) for control versus morphine groups, respectively. The 8-week volumetric analysis comparing control with morphine groups yielded callus volumes of 35.3 mm^3 versus 45.2 mm^3, respectively. Comparing the 4-week and 8-week postoperative control groups, there was a 29% decrease in callus volume from remodeling ($p = 0.023$). This extent of decreased volume and callus remodeling was not appreciated in the experimental morphine group. The morphine group callus volume only decreased 13% comparing 4-week with 8-week data ($p = 0.393$) suggesting a lack of callus remodeling and callus resorption in the experimental morphine arm (Fig. 3).

Morphine administration impaired the rate of callus maturation and delayed final osseous union. Qualitative evaluation of the histological sections was performed looking at the extent of fibrous tissue development, cartilage, woven immature bone and lamellar mature bone, callus consolidation and remodeling, and final osteotomy union. This evaluation revealed minimal differences between the groups at the 4-week postoperative point (Fig. 4). All sections being assessed as primarily immature callus had essentially equivalent levels of fibrous tissues, cartilage, and areas of woven bone. At 8 weeks postoperatively, there was a

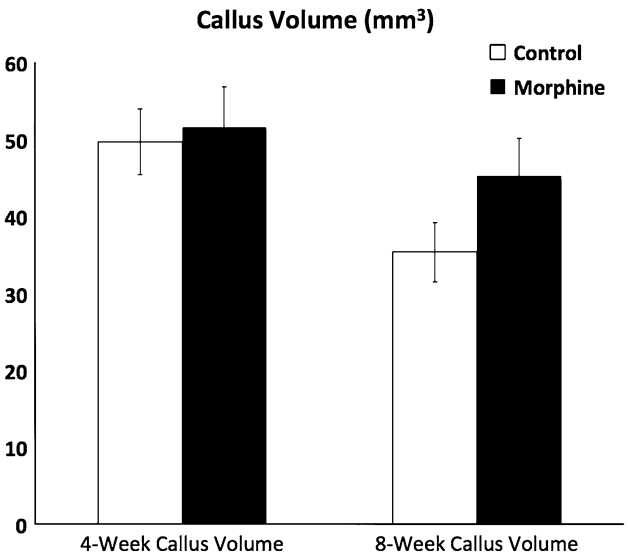

Fig. 3 Mean total callus volume ± SE at 4 weeks and 8 weeks quantitated by μCT and AMIRA software. Micro-CT analysis expressed remodeling and resorption as a decrease in callus volume over the two time points. The control group had significant levels of resorption decreasing 29% ($p = 0.023$) over the 4-week to 8-week time interval. Morphine administration inhibited callus resorption and remodeling with only a 13% decrease ($p = 0.393$) in callus volume comparing these time points.

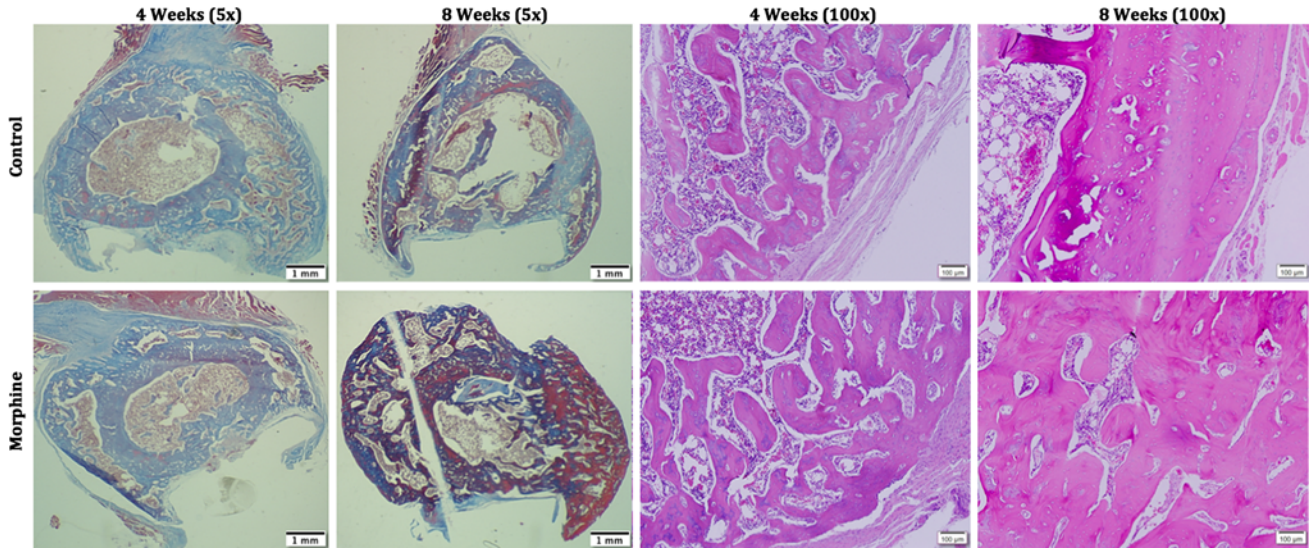

Fig. 4 Cross-sections with trichrome stain at ×5 magnification and H&E stain at ×100 comparing 4- and 8-week histology for the control and morphine groups. The 4-week groups are qualitatively similar with immature callus, areas of woven bone, and fibrous tissues. There were more differences noted at the 8-week comparison. The control animals both grossly at ×5 and microscopically at ×100 had a larger degree of consolidation, remodeling, and lamellar bone formation. This was not as evident in the 8-week morphine-treated animals. There was persistence of immature-appearing fibrous tissues, callus, and bone with minimal evidence of substantial remodeling.

notable difference with remodeling, consolidation, and evidence of lamellar bone identified within the control group samples. There was a lack of remodeling and a lesser degree of maturation of callus in the morphine group compared with the control group.

Discussion

Early and adequate acute pain control is essential in fracture and postsurgical care. The current mainstay for management in orthopaedic pain control is opioid analgesics with greater than 80% of patients with fracture receiving opioids [1, 10]. Little work has addressed the question of whether there is a relationship between fracture nonunion and opioid medications [2] or between increased use and duration of opioids in patients with tibial nonunions [1]. However, it remains unclear in those studies if opioid use and duration were increased from nonunion-associated pain or if opioids were a component, and potential driving force, for the nonunion. With rates of approximately 5% to 10% of patients having problems obtaining final union of their fractures [5, 14], the prevalence of this complication warrants continued investigation into this problem. Our results show a decrease in callus strength comparing control, saline-treated animals with morphine-treated animals. This is the first study to our knowledge to identify such a finding. Based on histology and μCT, the postulated mechanism for this finding is a retardation in the rate of callus maturation and remodeling in the morphine-treated animals.

There are several potential limitations to this study. First, it is an animal model. Further clinically based studies will be needed to verify if these results can be correlated to clinical practice. An animal model was chosen because this correlation has not to date been demonstrated or thoroughly studied in the existing literature. In the clinical setting, there are many potential impediments to osseous union (smoking, diabetes, nutrition, surgical intervention, compliance, etc) and isolating the effect of opioids seemed achievable only in a controlled experiment in an animal model. This work should be continued in the fracture and postoperative setting to further validate the clinical relevance of these findings. Second, this experiment concluded at 8 weeks postoperatively. It could be speculated that had the final euthanasia date been later, the experimental animal callus might have ultimately reached equal final strength as controls. Eight weeks was used because it has been a common end point in previous animal healing models, and clinically 6 to 8 weeks is a common time point at which the orthopaedic surgeon begins assessing fracture union. Further studies with later time points will be needed in the future to evaluate this limitation.

Morphine administration inhibited fracture callus strength when measured at 8 weeks postoperatively. This is the first study of its kind to isolate an opioid pain medication and associate it to impaired osseous union. There is emerging evidence that opioids do affect the skeleton by decreasing bone mineral density [7], increasing fracture risk in elderly patients with osteoarthritis [11], and increasing bone loss and incidence of spontaneous fractures in sarcoma models [9]; however, the mechanism is still in question. This animal

model suggests that inhibition of callus maturation leads to a biomechanically weaker bone in the subacute setting at 8 weeks postoperatively.

Qualitative and quantitative evaluation of the fracture callus showed inhibition of the maturation and remodeling in morphine-treated animals at 8 weeks postoperatively. Grossly, histologically and radiographically the 8-week morphine-treated animals had an abundance of callus formation and callus retention. The callus quality, however, was inferior biomechanically and on histologic and μCT assessment revealed a lack of maturation and remodeling, which was seen in the control saline-treated animals. These findings were found histologically as well as seen with advanced imaging on μCT scanning. Identifying that both histology and CT scans revealed similar and associated findings is significant, because this will allow for further investigation in the clinical setting.

We found that morphine administration results in weaker fracture callus strength by decreasing callus maturation and callus remodeling when measured at 8 weeks postoperatively in this animal model. The findings of this study may shed light on the etiology of some established nonunions. It may also push our current postoperative pain control regimens further toward multimodal treatments in an attempt to limit opioid narcotic consumption and may further drive development of new pain medications with narrower side effect profiles. More work on this topic is needed to identify the mechanism of this outcome and its relevance in the clinical setting.

Acknowledgments We thank Dr Kent Bachus for his guidance in developing an animal model and biomechanical expertise and Saranne Gross for her technical assistance throughout the duration of this study.

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