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Opioids Delay Healing of Spinal Fusion: A Rabbit Posterolateral Lumbar Fusion Model

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Abstract

Background Context

Opioid use is prevalent in the management of pre- and postoperative pain in patients undergoing spinal fusion. There is evidence that opioids downregulate osteoblasts in vitro, and a previous study found that

morphine delays the maturation and remodeling of callus in a rat femur fracture model. However, the effect of opioids on healing of spinal fusion has not been investigated before. Isolating the effect of opioid exposure in humans would be limited by the numerous confounding factors that affect fusion healing. Therefore, we have used a well-established rabbit model to study the process of spinal fusion healing that closely mimics humans.

Purpose

The objective of this work was to study the effect of systemic opioids on the process of healing of spinal fusion in a rabbit posterolateral spinal fusion model.

Study Design/Setting

This is a preclinical animal study.

Materials and Methods

Twenty-four adult New Zealand white rabbits were studied in two groups after approval from the Institutional Animal Care and Use Committee (IACUC). The opioid group (n=12) received 4 weeks' preoperative and 6 weeks' postoperative transdermal fentanyl. Serum fentanyl levels were measured just before surgery and 4 weeks postoperatively to ensure adequate levels. The control group (n=12) received only perioperative pain control as necessary. All animals underwent a bilateral L5–L6 posterolateral spinal fusion using iliac crest autograft. Animals were euthanized at the 6-week postoperative time point, and assessment of fusion was done by manual palpation, plain radiographs, microcomputed tomography (microCT), and histology.

Results

Twelve animals in the control group and 11 animals in the opioid group were available for analysis at the end of 6 weeks. The fusion scores on manual palpation, radiographs, and microCT were not statistically different. Three-dimensional microCT morphometry found that the fusion mass in the opioid group had a lower bone volume (p=.09), a lower trabecular number (p=.02), and a higher trabecular separation (p=.02) compared with the control group. Histologic analysis found areas of incorporation of autograft and unincorporated graft fragments in both groups. In the control group, there was remodeling of de novo woven bone to lamellar organization with incorporation of osteocytes, formation of mature marrow, and relative paucity of hypertrophied osteoblasts lining new bone. Sections from the opioid group showed formation of de novo woven bone, and hypertrophied osteoblasts were seen lining the new bone. There were no sections showing lamellar organization and development of mature marrow elements in the opioid group. Less dense trabeculae on microCT correlated with histologic findings of relatively immature fusion mass in the opioid group.

Conclusions

Systemic opioids led to an inferior quality fusion mass with delay in maturation and remodeling at 6 weeks in this rabbit spinal fusion model. These preliminary results lay the foundation for further research to investigate underlying cellular mechanisms, the temporal fusion process, and the dose-duration relationship of opioids responsible for our findings.

Keywords

Animal; Biology; Bone; Fusion; Healing; MicroCT; Narcotics; Opioids; Rabbit; Spine

Introduction

Opioid prescription for chronic back pain has risen at an enormous rate in the United States.[1,2] In patients with persistent and unrelieved symptoms of degenerative spine pathology, spinal fusion is an effective procedure for appropriately selected patients.[3,4,5,6,7,8,9,10] Consequently, up to 55% of patients are on chronic opioid therapy before an indication for spinal fusion and often continue to use opioids for postoperative pain during the healing of fusion.[1,11,12,13,14]

Failure of fusion healing remains a concern after spinal fusion as it can result in poor clinical outcome, need for revision surgery, and additional health-care costs.[15,16,17,18,19] Factors such as smoking, osteoporosis, obesity, diabetes, number of levels treated, use of instrumentation or interbody grafts, and surgical approach have been shown to influence fusion rates.[20,21,22,23,24,25,26,27,28] Whereas extensive preclinical and clinical studies have analyzed the effect of non-steroidal anti-inflammatory drugs (NSAIDs) on spinal fusion,[29] the effect of opioids has not been studied to date. The scientific rationale behind exploring the role of opioids is that they have been shown to have an inhibitory effect on osteoblasts in vitro,[30] and chronic opioid use leads to suppression of hypothalamic-pituitary-gonadal axis resulting in osteoporosis.[31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47] One previous study found that morphine delays the maturation and remodeling of callus in a rat femur fracture model.[48] Reproducing these findings in humans would be limited by the numerous confounding factors that affect fusion healing. Therefore, we have used a well-established rabbit model to study the process of spinal fusion healing that closely mimics that in humans.[49,50,51,52]

Given the widespread use of opioids in patients undergoing spinal fusion, identification of opioid use as a contributor to poor fusion healing will have important implications for providers and patients regarding pain management strategies. The primary objective of our study was to investigate the effect of systemic opioids on healing of spinal fusion in a rabbit posterolateral spinal fusion model.

Materials and methods

Animals

All procedures performed in the study were carried out after approval from the Institutional Animal Care and Use Committee (IACUC). Sample size estimation was done a priori at alpha=0.05 and 80% power to detect a 0.5-unit difference in fusion score between two groups. This estimated 20 animals and we studied 24 to account for attrition caused by death or exclusion of animals. Adult (1 year), male, 4.0- to 5.0-kg New Zealand white rabbits (*Oryctolagus* sp.) (Charles River Laboratories, Wilmington, MA, USA) were randomly assigned to control and opioid groups of 12 animals each.

Opioid group (n=12)

The animals received transdermal fentanyl 4 weeks preoperatively and 6 weeks postoperatively. A 25-µg/h fentanyl patch (Duragesic; Janssen Pharmaceuticals, Inc, Titusville, NJ, USA) was placed over the central auricular artery and changed every 72 hours for the study duration. Such a protocol has been shown to achieve a range of plasma fentanyl concentration (0.5–2.0 ng/mL) considered analgesic in humans.[53] Serum fentanyl levels were measured on the day of surgery before incision and at 4 weeks postoperatively to ensure the presence of fentanyl in this group. High-performance liquid chromatography-tandem mass spectrometry method was used to quantify serum fentanyl levels

(Thermo Accela UHPLC System with PAL autosampler and TSQ Discovery mass spectrometer; Thermo Fischer Scientific, Waltham, MA, USA).

Control group (n=12)

The animals in the control group received perioperative pain control consisting of a single fentanyl patch and NSAIDs for 3–5 days as required.

Surgical procedure

All animals underwent a bilateral L5–L6 posterolateral, intertransverse fusion with autologous iliac crest bone as previously described.[49,52] Through a dorsal midline skin incision and paramedian fascial incisions, the intermuscular plane between the multifidus and longissimus muscle was developed to expose the transverse process at L5 and L6 bilaterally. The transverse processes were decorticated, and morselized bone graft harvested from both iliac crests (1.0–1.1 g from each side) was laid over the intertransverse membrane. Morselized bone graft from each iliac crest was weighed on a high-precision scale in a sterile fashion before being laid down to ensure uniformity. Watertight closure of the fascia was done with 3-0 Vicryl (Ethicon US, LLC, Bridgewater, NJ, USA), and subcuticular skin closure was done with 3-0 Monocryl (Ethicon US, LLC, Bridgewater, NJ, USA). All animals were placed in individual housing during the postoperative period.

Outcome measures

All animals were euthanized 6 weeks postoperatively by lethal injection of intravenous pentobarbital (Euthasol; Le Vet Pharma BV, The Netherlands). Six weeks has been found adequate to study fusion healing in a rabbit intertransverse spinal fusion model using autograft.[50,52] The lumbar vertebral column from L1 to the sacroiliac joint was harvested en bloc, taking care not to disrupt the fusion mass. Fusion was assessed by manual palpation, plain radiographs, microcomputed tomography (microCT), and histology.

Manual palpation

Motion testing by a previously described protocol[54] was done at L5–L6 and the scores were compared with adjacent unfused segments. Fusion at L5–L6 was scored as 0, similar motion to adjacent unfused segments; 1, reduced motion but not rigid; and 2, rigid with no motion. A score of 0 or 1 was considered as not fused, and a score of 2 was considered as definite fusion.

Radiographs

Anteroposterior plain radiographs (Faxitron A43855A, Hewlett-Packard, Palo Alto, CA, USA) were evaluated blindly by two spine surgeons according to a grading scale described previously.[55] Fusion was scored as 4, intertransverse bone mass present bilaterally without lucency; 3, bone mass bilaterally with lucency on one side; 2, bone mass bilaterally with bilateral lucencies; 1, bone mass only on one side; and 0, no bone mass on either side. Definite fusion was considered present in specimens who had a score of 3 or 4. A score of ≤2 was taken as not fused.

MicroCT

Microcomputed tomography was performed using a GE eXplore system (GE Healthcare, London, Ontario, Canada). The image acquisition parameters were as follows: power=80 kV, current=450 µA, exposure time=400 ms, pixel size=45 µm, number of excitations=2, and angle of increment 0.4. All

images were reconstructed as Digital Imaging and Communications in Medicine using the GE eXplore software (GE Healthcare). Two- and three-dimensional analyses were performed using the commercially available Bruker Skyscan microCT software (Bruker microCT, Kontich, Belgium). Additional details on microCT methodology can be found in Supplementary Appendix SA1. Three-dimensional reconstructed microCT images were graded for fusion in a blinded fashion using the same score used for plain radiographs. Microcomputed tomography scanning is used extensively and is considered the gold standard for the assessment of bone microarchitecture and density.[56,57,58,59,60,61,62] The following three-dimensional morphometric parameters were calculated from the developing fusion mass and compared between study groups: bone volume, trabecular thickness, trabecular separation, trabecular number, and bone mineral density.

Histology

Lumbosacral spine specimens were fixed in 10% neutral-buffered formalin for 72 hours at room temperature. Specimens were cut to isolate the operated L5–L6 segment, after which they were decalcified (Surgipath Decalcifier 1; Leica Biosystems Inc, Buffalo Grove, IL, USA). Colored dyes were used to identify the right and the left sides. Five-millimeter sections were trimmed medially, centrally, and laterally from the fusion mass on both sides, which subsequently underwent paraffin embedding. Four-micrometer tissue sections were prepared using an automated microtome (Leica RM2255, Leica Biosystems Inc) and were placed on electrostatically coated microscope slides. The sections were stained with Mallory aniline blue connective tissue stain and were examined under light microscopy. The sections were evaluated without the knowledge of the groups (ie, blinded) to describe the process of new bone formation.

Statistical analysis

The presence or the absence of fusion on manual palpation, radiographs, and microCT has been expressed as number and percentage. Fusion was recorded as definite only when both blinded observers scored a fusion. Quantitative manual palpation score, radiographic fusion score, and microCT parameters were compared by the Student *t* test and interpreted at a significance level of $p<.05$.

Results

Twelve animals in the control group and 11 animals in the opioid group were available for analysis at the end of 6 weeks. All animals tolerated the surgery well and were mobile in the postoperative period. One animal in the opioid group was removed prematurely because of a progressive weight loss of >20% despite nutritional augmentation. One animal in the control group developed a superficial skin abscess in the interscapular region away from the surgical site and was treated successfully with antibiotics.

Serum fentanyl levels

The mean serum fentanyl level in the opioid group at preoperative assessment (before skin incision) was 2.73±0.24 ng/mL. At 4 weeks postoperatively, the mean level was 1.58±0.71 ng/mL.

Assessment of fusion

On manual palpation, 9 of the 12 specimens (75%) in the control group and 8 of the 11 specimens (72.7%) in the opioid group were scored as fused (score of 2). The remaining three specimens in each group were scored as 1 (reduced motion but not rigid). Plain radiographs were scored as fused in 8 of the 12 specimens (66.7%) in the control group and in 7 of the 11 specimens (63.6%) in the opioid group

(Fig. 1). Three-dimensional reconstructed microCT images were determined as fused in 7 of the 12 specimens (58.3%) in the control group and in 7 of the 11 specimens (63.6%) in the opioid group (Fig. 2, Fig. 3). The mean fusion scores were not significantly different between the opioid and the control groups (Fig. 4).

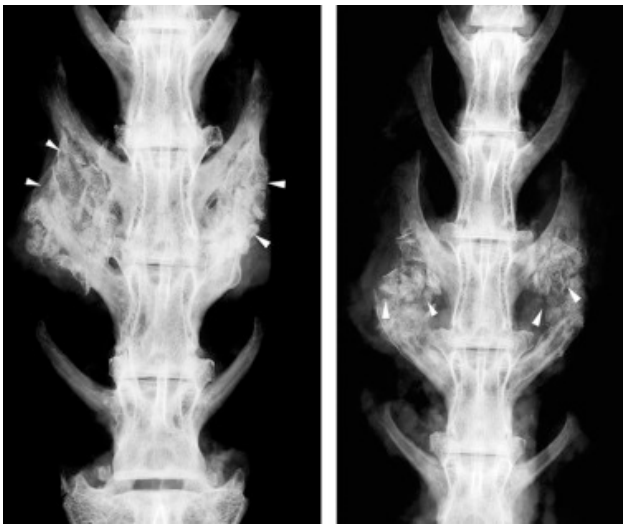

Fig. 1. Representative anteroposterior plain radiographs of rabbit lumbar spine specimen. (Left) Bridging fusion mass seen with no lucency (fusion score of 4). (Right) Fusion mass seen with bilateral lucency (white arrowheads) (fusion score of 2).

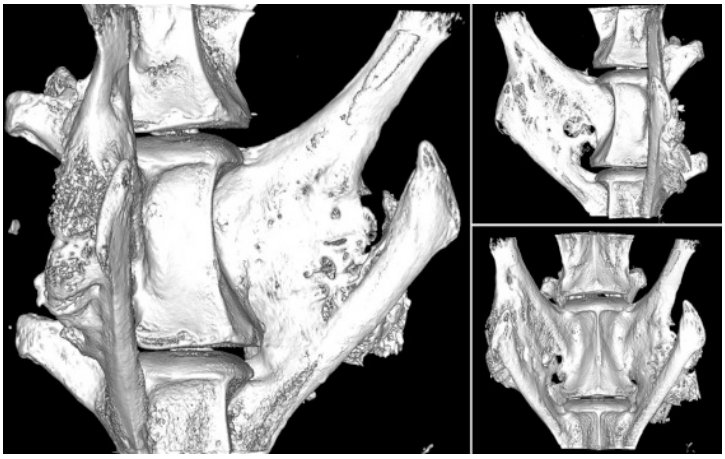

Fig. 2. Representative three-dimensional reconstructed microCT scan of rabbit lumbar spine specimen showing bilateral bridging fusion with no lucency (fusion score of 4): left oblique view (Left), right oblique view (Top Right); anteroposterior view (Bottom Right). microCT, microcomputed tomography.

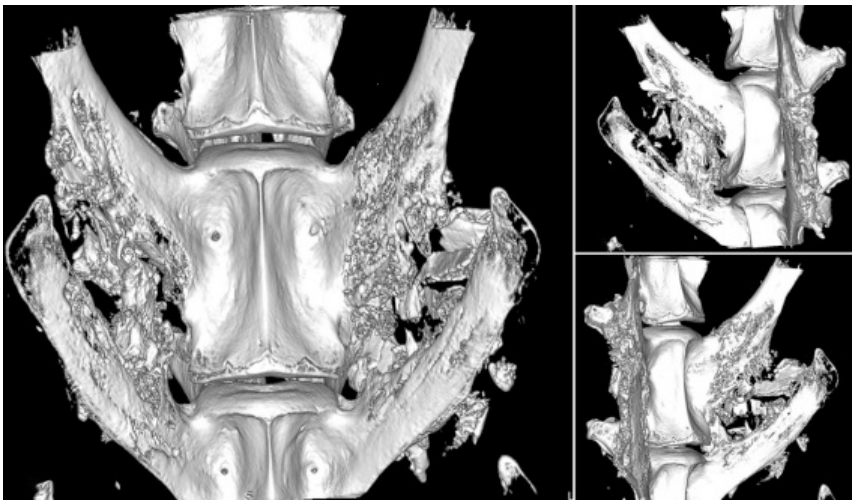

Fig. 3. Representative three-dimensional reconstructed microCT scan of rabbit lumbar spine specimen showing bilateral fusion mass with lucency and unresorbed autograft fragments (fusion score of 2): anteroposterior view (Left); right oblique view (Top Right); left oblique view (Bottom right). microCT, microcomputed tomography.

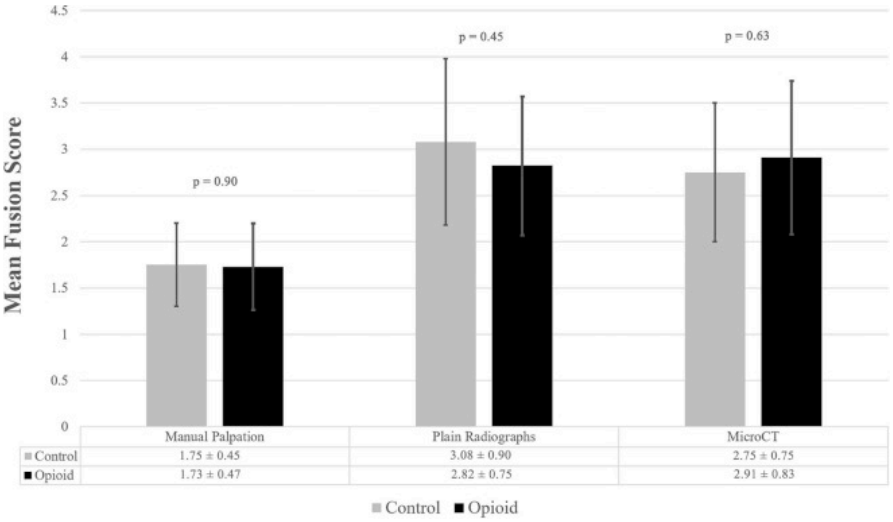

Fig. 4. Mean fusion score on manual palpation, plain radiographs, and microCT analysis between control (n=12) and opioid (n=11) rabbit groups. microCT, microcomputed tomography.

Microcomputed tomography analysis showed that the mean bone volume of the fusion mass was lower in the opioid group (679±122 mm^3) than in the control group (742±125 mm^3). Although trending toward significance, the p-value was .09 on statistical comparison. Fusion mass in the opioid group had a lower mean trabecular number, which was statistically significant (p=.02) compared with the fusion mass in the control group. Additionally, the mean trabecular separation was higher in the opioid group compared with the control group, which was statistically significant as well (p=.02). The mean bone mineral density was slightly lower in the opioid group but statistically insignificant (Table).

Table. Results of the three-dimensional morphometric analysis of intertransverse fusion mass by microCT

	Control group (n=24*)	**Opioid group (n=22*)**	**p-Value**
Bone volume (mm)	742±125	679±122	.09
Trabecular thickness (mm)	0.73±0.11	0.74±0.11	.76
Trabecular number (mm^{-1})	0.103±0.016	0.092±0.014	.02†
Trabecular separation (mm)	5.14±0.60	5.6±0.76	.02†
Bone mineral density (mg/cc)	0.783±0.024	0.771±0.041	.22

* Bilateral measurement of intertransverse fusion mass in each animal.

† Statistically significant.

Histology

Microscopic analysis of fusion in the control and the opioid groups demonstrated new bone formation with incorporation of autograft fragments within the fusion mass. Most of the sections showed 75%–100% of the fusion mass composed of new bone, and some sections showed up to 20% of cartilage and fibrous tissue (Fig. 5). This composition of elements was similar in the control and the opioid groups. The developing fusion mass in both groups consisted mainly of intramembranous ossification and few foci of endochondral ossification. Areas of incorporation of autograft and unincorporated graft fragments were seen in both groups. In the control group, there was remodeling of woven bone to lamellar organization with incorporation of osteocytes, formation of mature marrow, and relative paucity of hypertrophied osteoblasts lining new bone (Fig. 6). Sections from the opioid group showed formation of woven bone, and hypertrophied osteoblasts were seen lining the new bone. There were no sections showing lamellar organization and development of mature marrow elements in the opioid group (Fig. 7). These findings indicate a delay in the remodeling and maturation process of new bone in the opioid group compared with the control group.

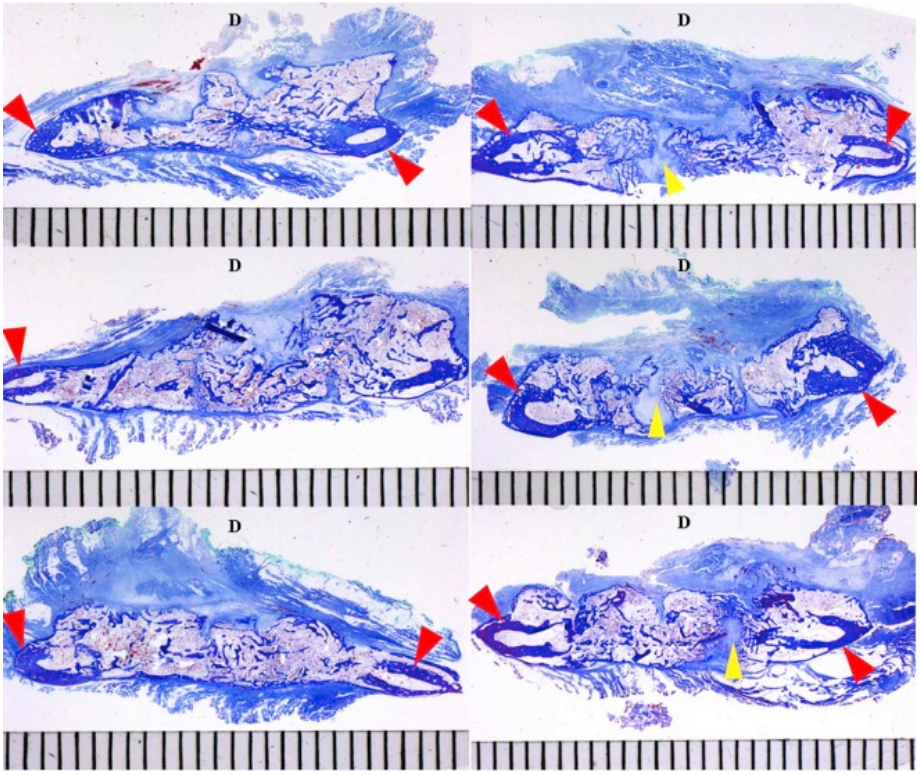

Fig. 5. Micrograph of sagittal intertransverse fusion sections. (Left) One hundred percent bridging bone between adjacent transverse processes (red arrowheads). (Right) Incomplete bridging of bone with intervening cartilage (yellow arrowheads) between adjacent transverse processes (red arrow). Mallory aniline blue connective tissue stain, millimeter scale in the field. D, dorsal; microCT, microcomputed tomography.

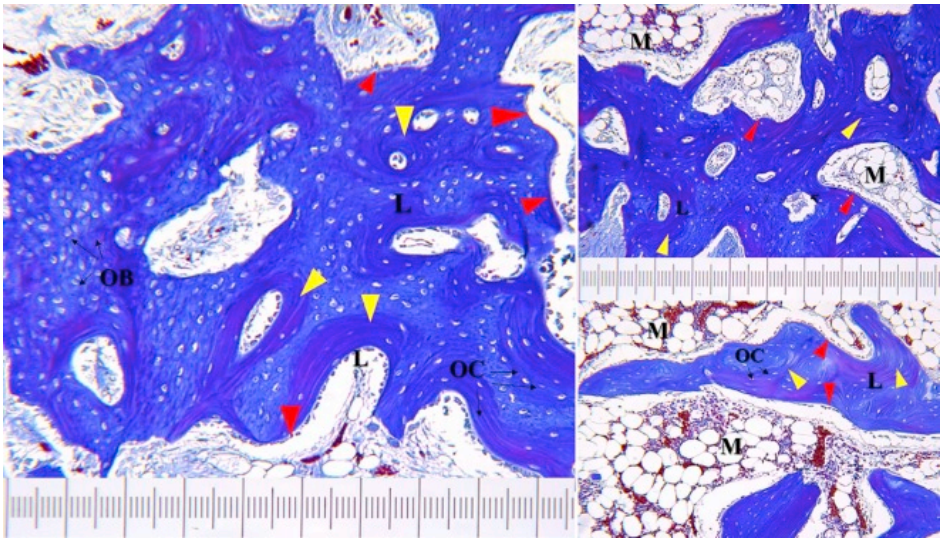

Fig. 6. Representative micrograph of fusion in control group showing W bone undergoing remodeling and maturation of intramembranous W bone. (Left, Top Right, and Bottom right) Lamellar organization of matrix (yellow arrowheads) with incorporated OCs. Small and less dense OBs are seen lining new bone (red arrowheads). Developing M elements. (Left) Area of W bone with haphazard arrangement of incorporated OBs and unorganized matrix can be seen. Compare with smaller and tapered OCs in the organized L matrix. Mallory aniline blue

connective tissue stain, ×79 magnification, 10 µm/smallest division scale in the field. OB, osteoblast; L, lamellar; OC, osteocyte; M, mature marrow; W, woven.

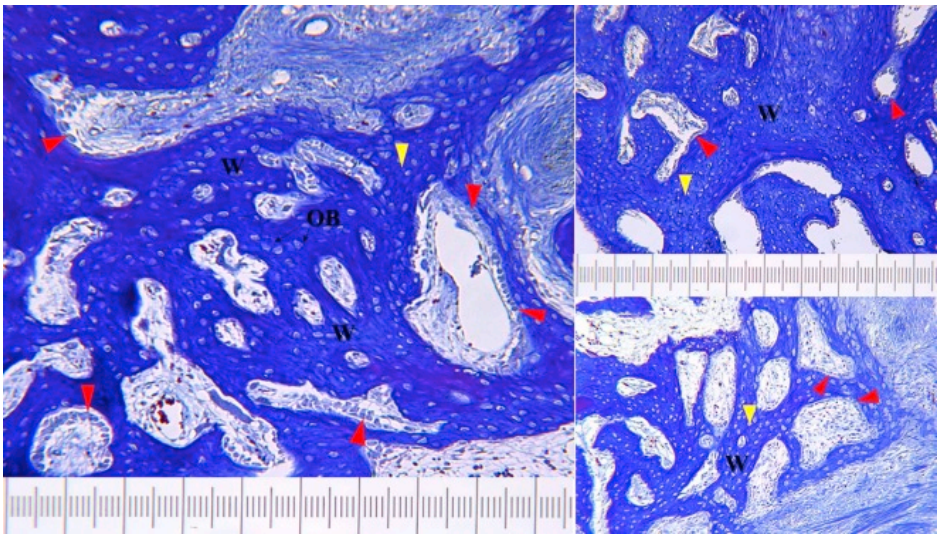

Fig. 7. Representative micrograph of fusion in the opioid group showing intramembranous W bone. (Left, Top Right, and Bottom right) Woven bone (yellow arrowheads) with haphazard arrangement of incorporated OBs and unorganized matrix can be seen. Hypertrophied and dense OBs seen lining new bone (red arrowheads). Mallory aniline blue connective tissue stain, ×79 magnification, 10 µm/smallest division scale in the field. W, woven; OB, osteoblast.

Discussion

Although many technological advancements in spinal implants, instrumentation, and biologics have reportedly improved the rates of spinal fusion, these advances come at considerable costs to the health system.[63,64,65,66,67,68,69,70] Optimization of modifiable patient factors before spinal fusion probably represents a cost-effective way to improve the chance of fusion success. A biologically plausible and modifiable exposure that has not been studied in spinal fusion is opioid use. Our study, which uses a rabbit posterolateral spinal fusion model, is the first to investigate the effect of opioids on the healing of spinal fusion. We found that the presence of systemic opioids in the pre- and postoperative periods negatively affects the process of spinal fusion healing. Fusion mass in animals with opioid exposure had fewer and widely spaced trabeculae on microCT analysis. Additionally, there was a delay in the maturation of woven bone on histologic analysis in the opioid group. These findings indicate a less mature and inferior quality fusion mass because of opioids.

Fentanyl is a Schedule II controlled substance with a predominant mu-opioid receptor action like other widely prescribed opioids, such as hydrocodone, oxycodone, and hydromorphone.[71] Our protocol consisted of 4 weeks' preoperative and 6 weeks' postoperative opioid exposures. We have also confirmed adequate levels of systemic fentanyl at two different time points in the animals in the opioid group. Therefore, opioid administration in our study group closely resembles real-life clinical opioid use and supports the validity of our analysis.

Assessment of fusion on manual palpation, plain radiographs, and microCT did not reveal a significant difference between the opioid and the control groups. Three-dimensional volumetric analysis on microCT revealed a smaller mean fusion mass in the opioid group than in the control group, although

not statistically significant. The mean number of trabeculae per unit length of fusion mass was significantly lower in the opioid group than in the control group. Accordingly, the mean separation between the trabeculae was significantly higher in the opioid group fusion mass. These findings suggest a less dense trabecular network of the newly formed bone. Whereas new bone formation was seen in both the control and the opioid groups on histologic analysis, remodeling and maturation of woven bone were delayed in the opioid group. In the control group, there was an organization of matrix with incorporation of osteocytes and formation of mature marrow elements. These changes are consistent with the expected process of healing in rabbit posterolateral spinal fusion with autograft at 6 weeks.[52] In the opioid group, there was woven bone formation with no lamellar organization and no appearance of marrow elements. Findings on microCT corroborate with the histologic findings of relatively immature and less dense trabeculae of the fusion mass in the opioid group. Additionally, intact processes of initial fusion healing (woven bone) could be the reason for no appreciable difference in radiographic fusion between our study groups.

Prior research has demonstrated opioid receptors (mu, delta, and kappa) on human osteoblast-like cell lines [30]. Osteocalcin, which is a marker of osteoblast activity, has been shown to be reduced significantly by opioids.[30,31,32] Previously, a rat femur fracture model was used to study the effect of opioids on bone healing. The authors found decreased callus strength at 8 weeks in rats exposed to morphine postoperatively compared with control. Also, callus maturation and remodeling was delayed from the 4- to 8-week time point in the morphine group.[48] Abundant preclinical and clinical data show that chronic opioid use inhibits functioning of the hypothalamic-pituitary-gonadal axis, resulting in hypogonadism and osteoporosis.[31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47] Not only does osteoporosis increase the risk of fracture but also it is an important risk factor for pseudoarthrosis after spinal fusion.[72,73,74] Therefore, there is a scientific rationale to incriminate opioids as a risk factor for poor fusion healing, but there is a need for more evidence. We believe our findings are consistent with the previously investigated role of opioids in bone healing and lay the foundation for further research. If conclusively proven, our findings will have important implications in the clinical setting. A negative effect of opioids on fusion remodeling can be counterproductive to augmentation of fusion by instrumentation, interbody cages, and biologics. As remodeling of fusion is an ongoing process, opioid use can affect final maturation and strength of fusion despite no obvious effect on the radiographic evidence of fusion.

We report our results with limitations. The present study uses a preclinical spinal fusion model and findings from an animal model may not necessarily translate to clinical scenarios directly. However, the rabbit posterolateral spinal fusion model has been validated to closely resemble healing of spinal fusion in humans.[49,50,51,52] Additionally, isolating the effect of opioids in clinical studies will be particularly difficult, given the numerous confounding medical and surgical variables affecting fusion, highlighting the importance of evidence from animal models. We did not explore the underlying cellular mechanisms (osteoblast, osteoclast, and osteocyte functions, growth factors and signaling, gene expression, etc.) that could be responsible for our findings. We have not performed biomechanical testing to quantify the strength of fusion. We have studied a single dose and duration of fentanyl in the opioid group and are unable to comment on the possibility of a dose-duration response of opioids. Lastly, we have not studied the temporal process of fusion. It may be possible that fusion catches up over extended follow-up; however, even knowledge of delay in healing will be important as opioid use is potentially modifiable. Also, in the presence of other patient risk factors such as smoking, diabetes, and osteoporosis, the additive risk of opioid use can be significant. Despite these limitations, ours is the first

in vivo study exploring the effect of opioids on healing of spinal fusion and provides avenues for further research.

In conclusion, our findings raise concern, albeit preliminary, that opioid use delays the healing of spinal fusion. Additional preclinical and clinical studies will be required to confirm our initial findings. In addition to addressing our current limitations, future research may benefit from investigating the influence of opioid type, dose, and duration. Opioid formulations with less potent mu-receptor action (buprenorphine) or dual mechanism (tapentadol) have less impact on bone than potent mu-receptor opioids such as morphine, fentanyl, and hydrocodone.[33,42] Whether a preoperative opioid "washout" period or delaying the use of opioids postoperatively is beneficial are questions that will be crucial to answer. Extensive research on the effect of NSAIDs on spinal fusion has led to widespread changes in practice patterns relating to its use.[29] The knowledge of such an effect caused by opioids will be important not only for spine surgeons but also for patients undergoing spinal fusion. A higher risk of delayed or suboptimal healing of fusion and the need for revision surgery have the potential to add significant morbidity and health-care costs.

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Supplementary material

The following is the supplementary data to this article:

https://ars.els-cdn.com/content/image/1-s2.0-S1529943018301669-mmc1.docx

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