Effect of local zoledronic acid administration in a rat model of posterolateral spinal fusion

Ashley Strickland, Daniel Cavanaugh, William H. Leatherwood*, James Raynor, Alexander Brown, Paul S. Weinhold

University of North Carolina School of Medicine, 134 Glaxo Bldg., 101A Mason Farm Rd., Chapel Hill, NC, 27599, USA

ARTICLE INFO

Keywords:

Spinal fusion
Zoledronic acid
Bone autograft
Bisphosphonate
Rats

ABSTRACT

Study design: Randomized animal model study.

Purpose: Posterolateral spinal fusion represents a common surgical procedure in the United States. The effect of bisphosphonate administration in these patients is poorly understood. The purpose of this study is to determine whether local administration of bisphosphonate by soaking bone autograft would affect the apparent bone density or structural properties of the fusion mass in a rat model of posterolateral spinal fusion.

Methods: 36 Spring Dawley rats underwent L4-5 posterolateral spinal fusion with bone autograft. These rats were divided into three groups, two experimental groups and one control group. Each of the experimental groups underwent spinal fusion with morselized vertebral cortical and cancellous autograft soaked in zoledronic acid solution; one group 20 mcg/mL, another 200 mcg/mL. The control group underwent L4-5 spinal fusion with cancellous allograft soaked with saline. At 8 weeks, the rats were euthanized for analysis. Evaluations consisted of micro-CT scanning, four-point bending biomechanical testing, histology, and radiographs.

Results: Both of the experimental groups showed statistically significant increase in apparent bone density and bone volume fraction at the fusion mass. Biomechanical measures revealed a trend for improvement in the experimental groups, but these did not reach statistical significance.

Conclusions: This data suggest that locally administered bisphosphonate medications result in increased apparent bone density and bone volume fraction at the fusion mass in posterolateral spinal fusion, and that there appear to be no deleterious consequences with regards to the stiffness or maximum load to failure of the fusion mass under flexion bending evaluation.

1. Introduction

Spinal fusion is a common orthopaedic procedure, representing more than 250,000 procedures annually in the United States. Successful fusion relies on the formation of a solid fusion mass mediated by osteoblast-osteoclast interaction. While a common procedure, there are high rates of complications such as inadequate fusion and pseudoarthrosis occurring in 10–30% of cases. These complications often lead to chronic pain and the need for re-operation.¹ High complication rates from such a common procedure suggest the need for additional therapies to promote improved spinal fusion.

Bisphosphonates are a class of medications commonly used to treat metabolic bone disorders, such as osteoporosis which affects over 10 million patients in the United States. Bisphosphonates are known anti-resorptive medications with a 3-fold mechanism. They work to inhibit the process of osteoclastogenesis in the bone marrow, they decrease the

activity of osteoclasts on the bone surface, and also decrease the lifespan of osteoclasts by increasing the apoptosis rate of this cell line.² While this class of medications has been extensively studied in regards to osteoporosis, the clinical implications of this process on spinal fusion is poorly understood. There is growing literature to suggest that in addition to their anti-resorptive properties, these medications have a positive effect on bone formation and differentiation of osteoblastic precursor cells as well.^{2–4} This finding has led several researchers to investigate the potential effects of bisphosphonate-induced bone formation in the face of orthopaedic procedures. The majority of this research has been in the area of orthopaedic implant-bone interfaces, and several of these studies have shown that locally administered bisphosphonates have a positive impact on peri-implant bone formation.^{5–7}

The targets of action of these medications and the early promising results in the implant literature have motivated some to ask if they may have an effect on spine fusion. Thus far, there has been very little

* Corresponding author.

E-mail addresses: william_leatherwood@med.unc.edu, paul_weinhold@med.unc.edu (W.H. Leatherwood).

<https://doi.org/10.1016/j.jor.2019.08.021>

Received 13 July 2019; Accepted 12 August 2019

Available online 13 August 2019

0972-978X/ © 2019 Professor P K Surendran Memorial Education Foundation. Published by Elsevier B.V. All rights reserved.

investigation in the local delivery of these agents in the spine fusion area. We feel a better understanding of these medications is important for at least two reasons. First, these medications are becoming increasingly utilized in the general population, and many of these patients will undergo spinal fusion at some point in the future, and improved understanding of the local effect of these medications will aid in clinical decision making for these patients. The systemic use of bisphosphonates, specifically zoledronic acid (ZA) has been studied in animal models of spinal fusion, and have shown that this medication may have a positive impact on spinal fusion rates and fusion mass quality.^{8,9} Additionally, several studies have shown favorable results with regards to bony ingrowth into orthopaedic implants with local bisphosphonate administration, which is advantageous to limit possible systemic toxicity.^{5–7} It was our goal and.¹⁰ Our hypothesis was that local administration of ZA would promote improved spinal fusion as demonstrated by increased fusion mass apparent density by micro-CT and increased stiffness and strength under flexion bending evaluation.

2. Materials and methods

All experimental procedures were approved by the University of North Carolina (UNC) Institutional Animal Care and Use Committee (IACUC) prior to initiation of the study. Thirty-six female Sprague Dawley retired breeder rats (Envigo Inc., Dublin, VI) with a mean age of 24 weeks were randomly assigned to one of three treatment groups to give equivalent mean body weights of 320 g per group. Treatment groups included: 1) Control: saline solution, 2) ZA20: 20 mcg/mL ZA solution, 3) ZA200: 200 mcg/mL ZA solution. These dosages are within the range which other studies have shown a clinically significant treatment effect with local administration.¹¹ Rats underwent a L4-L5 posterolateral spinal fusion using 0.4 g of morselized caudal tail vertebrae soaked in 0.1 mL treatment solution serving as the graft source. Rats were housed in pairs in a registered and accredited USDA Animal Research facility and given *ad libitum* access to food and water with a 12-h light/dark cycle (7 a.m.–7 p.m.) throughout the study. Animals were followed for eight weeks before being euthanized and spines carefully dissected out for primary evaluations with micro-CT imaging and biomechanical testing. Secondary evaluations included qualitative histology and radiographs.

2.1. Surgical technique

Under isoflurane anesthesia, the tail was amputated, and cortical and cancellous autograft was harvested from four to five tail vertebrae to serve as the graft source. The tail incision was then closed with deep dermal 3-0 absorbable suture. The bone autograft was allowed to soak in one of the three treatment solutions for approximately 15 min prior to implantation. Following graft harvest, a posterolateral spinal fusion was performed through a bilateral Wiltse muscle splitting approach to the transverse processes of L4 and L5 utilizing a single dorsal skin incision, as described by Wei Yuan and colleagues.¹⁰ The transverse processes of L4 and L5 were decorticated bilaterally using a high-speed burr. The graft was then split into two aliquots and placed at each of the fusion sites. The muscle incisions were then closed using 3-0 absorbable suture in order to secure the graft in place. The skin was then closed with 3-0 absorbable deep dermal sutures followed by wound clips. Each rat received an immediate post-operation (post-op) x-ray to ensure correct placement. Pain control was controlled with twice daily injections of buprenorphine (0.3 mg/kg) for three days and *ad libitum* access to acetaminophen-doped drinking water (1.6 mg/mL) for seven days post-surgery. The rats were weighed weekly and monitored for complications before being euthanized at eight weeks for analysis. Additional x-rays were taken immediately following euthanasia for qualitative assessment of fusion mass.

2.2. Micro-computed tomography analysis (micro-CT)

Prior to biomechanical analysis, spines were dissected out from L3 to L6 with the surrounding soft tissues undisturbed. 30 specimens were then immersed in normal saline within a specimen tube in preparation for imaging. The remaining six were excluded as they were fixed in formalin and placed in 70% ethanol for histology. Micro-CT was obtained using an *in vivo* micro-CT system (eXplore CT120, GEHealthcareInc., Waukesha, WI) at a voxel resolution of 50 μ m in the UNC Biomedical Research Imaging Center. In the image scans the fusion mass was isolated bilaterally from the superior aspect of the L4 vertebral transverse process (TP) to the inferior aspect of the L5 TP. Only the area lateral to the pars interarticularis was analyzed as part of the fusion mass. This CT imaging data was then used to calculate fusion mass tissue volume (TV) and bone volume (BV) to give a BV fraction (BV/TV), and apparent density in mg HA/mL. Bone volume was segmented with a common threshold of 460mgHA/cc.^{12,13}

2.3. Four-point bending

Following micro-CT imaging, the same 30 specimens underwent biomechanical analysis utilizing the four-point bending technique described by Robinson and colleagues.¹⁴ The specimens were dissected taking care not to disrupt the fusion mass, facet capsules, ligaments, and intervertebral discs. The L3-L6 segments were then potted in an acrylic resin within two-inch-long segments of one-inch aluminum square tubing. These specimens were then loaded at 3 mm/min until failure or to a final actuator displacement greater than 2 mm under four-point bending loading using a servohydraulic materials testing system (8500 Plus; Instron, Norwood, MA). The inner and outer spans of the bending fixture were 25 and 60 mm respectively. The contact points were 5 mm stainless steel rollers. Each potted spine was tested using the four-point bending apparatus generating a vertical load versus deflection curve, which was then utilized for our analysis. The spines were loaded in flexion, as this would be the primary direction of stress in an *in vivo* situation. Data analyzed included stiffness of the fusion prior to 1 mm of displacement, and maximum load and energy prior to failure or 2 mm of displacement.

2.4. Histology and X-Rays

Two specimens from each group were fixed in 10% neutral buffered formalin and demineralized using a formic acid solution (Immunocal, StatLab, McKinney, TX). The specimens were then imbedded in paraffin and sectioned sagittally for histologic analysis. Slides were stained with Toluidine blue, digitized and viewed utilizing Leica Aperio Image Scope V-12.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the CT imaging data followed by multiple comparison testing using the Holm-Sidak method. The four-point bending data was not normally distributed, and was analyzed utilizing a Kruskal-Wallis analysis of ranks followed by Dunnett's multiple comparison testing to analyze for statistical differences between the test groups. Differences were considered significant if the p-value was less than 0.05 for all statistical analyses. Statistical analysis software was utilized to perform all calculations (SigmaPlot v11.0; Systat Software, San Jose, CA). A minimal clinically important difference was considered a 30% improvement in the measure relative to the control values. A power analysis determined that a sample size of 8 animals per group would be required to detect a 30% improvement in fusion stiffness in comparing 3 groups with a significance level of 0.05 and power of 0.8 in utilizing previously published standard deviation data for four-point bending evaluation of fusion stiffness with this model.¹⁵

Fig. 1. Representative 8-week post-op plain film radiographs. Left to Right: Control, ZA20, ZA200.

3. Results

All 36 rats who underwent spinal fusion survived the surgery and to the end of the experiment. No animals were excluded from analysis.

3.1. X-rays

A qualitative view comparing immediate post-op and 8-week post-op spine plain film radiographs appears to demonstrate more consolidation at the fusion site in the experimental groups compared to the control (Fig. 1).

3.2. Micro-CT results

The mean BV fraction for each group was: control: $63.2 \pm 24.6 \text{ mm}^3$, ZA20: $174.9 \pm 42.8 \text{ mm}^3$, and ZA200: $224.3 \pm 49.5 \text{ mm}^3$. The BV fraction of both experimental groups were significantly higher than the control group ($p < 0.001$). The BV fraction was also significantly increased in the ZA200 group when compared to the ZA20 group ($p < 0.01$). The apparent density for each group was: control: $303.0 \pm 41.7 \text{ mgHA/mL}$, ZA 20: $412.6 \pm 45.8 \text{ mgHA/mL}$, and ZA 200: $444.0 \pm 31.6 \text{ mgHA/mL}$ (Fig. 2). The apparent density of both experimental groups were significantly higher than the control group ($p < 0.001$). There were no significant difference comparing the apparent density between ZA20 and ZA200.

Fig. 2. Quantitative micro-CT analysis performed 8 weeks after L4-5 posterolateral fusion, fusion mass density (mgHA/mL). p-value: Control vs ZA20 < 0.001 , Control vs ZA200 < 0.001 , ZA20 vs ZA200 = 0.091. * Significant difference from control ($P < 0.05$).

3.3. Biomechanical analysis

Mean stiffness (N/mm) prior to 1 mm displacement were: control group 5.44 ± 2.43 , ZA20 group 9.33 ± 7.18 , and ZA200 group 11.83 ± 15.88 . There was not a significant difference detected in stiffness prior to 1 mm displacement among the groups ($p = 0.21$). Maximum energy (mJ) prior to 2 mm displacement were: control: 10.85 ± 2.38 , ZA20: 15.34 ± 6.40 , and ZA 200: 15.11 ± 6.35 . There was not a significant difference detected in energy prior to 2 mm displacement among the groups ($p = 0.08$). Maximum load (N) prior to 2 mm displacement were: control: 12.10 ± 3.41 , ZA20: 17.70 ± 8.34 , and ZA200: 17.80 ± 7.50 . There was not a significant difference detected in maximum load prior to 2 mm displacement compared to the control ($p = 0.10$). Each of the metrics evaluated demonstrated a difference between experiment groups and control that was more than the defined minimal clinically important difference, however none reached statistical significance. (Fig. 3).

3.4. Histology

Qualitative viewing of toluidine blue stained specimens from each group revealed evidence of new bone formation with osteoblastic rimming of bone fragments in all specimens. The presence of multinucleated giant cells was noted in all specimens, indicating that bony remodeling was taking place. The presence of fibrous tissue was noted in all groups as well. In the ZA200 specimens, there were areas of fibrous tissue surrounding un-remodeled bone fragments (Fig. 4).

4. Discussion

The results of our study indicate that locally administered zoledronic acid has a positive effect on posterolateral spinal fusion in a rat model. Administration of ZA results in an increased apparent density and BV fraction of the fusion mass, which may be dose-dependent with a trend towards increasing density with increasing dose. While our biomechanical measures did not reach statistical significance, local ZA does appear to positively affect fusion strength as demonstrated by an increasing trend in all biomechanical evaluations that was above our defined minimal clinically important difference. The design of our study does not allow us to comment on the fusion rate of each treatment group, and the fact that our data was not normally distributed, indicates that there were likely non-unions in all groups. However, the increasing trend in stiffness indicates that ZA does not negatively affect fusion.

Review of the histology does show that a higher ZA dose may affect bony remodeling, as there is evidence of un-remodeled bone surrounded by fibrous tissue in our ZA200 group (Fig. 4). However, this did not appear to have a negative effect on the biomechanical stiffness of these specimens as there was a trend towards increasing stiffness with increasing ZA dose. These results are similar to the reported

Fig. 3. Four-point bending analysis data following posterolateral fusion. Left to right: linear stiffness prior to 1 mm displacement (N/mm), maximum load prior to 2 mm of displacement (N), and energy imparted prior to 2 mm of displacement (mJ). Dashed line = mean, solid line = median, box = upper and lower quartile.

histology results reported by Yasen and colleagues with systemic administration of ZA.⁸ They also reported a higher fusion rate in rats who were treated with high-dose systemic ZA therapy.

Our model was unique compared to the other animal studies in the literature, as most studies have focused on systemic bisphosphonate administration while we used local. Local administration would be an attractive option, as it could be administered as a one-time dose at the time of surgery. In addition, local administration would theoretically decrease the risk of other drug side-effects.

Going forward, we would propose several potential improvements to our study protocol. The first of these would be to evaluate the fusion at a more extended time point when a greater number of the fusion sites had fused thus reducing the variability in the fusion stiffness measure. The improvements in four-point bending measures may become significant with a reduction in the variability of biomechanical measures that may occur with more well healed fusions. Also, if there is a transient drug-induced delay in remodeling, euthanizing rats across a spectrum of time points may establish that once the treated fusion masses are remodeled, treatment with ZA may result in a larger and more robust fusion mass. This would be important to understanding the clinical utility of bisphosphonate therapy in spine surgery. It may also be beneficial to include an experimental group treated with systemic ZA to see if there is a difference in the medication effect based on delivery method. In addition, varying the bone graft source may result in a different treatment effect. We chose to use tail autograft in order to approximate the use of local vertebral autograft commonly used in spine surgery. Iliac crest autograft and allograft are also commonly utilized in spine surgery. As zoledronate's proposed mechanism for stimulating bone formation is driving marrow derived stem cells into an osteogenic lineage, it may prove beneficial to use the iliac crest as a graft source due to the higher prevalence of stem cells compared to tail vertebrae.

5. Conclusion

In our rat model, the local administration of ZA promotes the formation of a more robust fusion mass demonstrated by significantly increased apparent density and BV fraction. Flexion bending

biomechanical data revealed improvements with ZA administration that exceeded what would be considered a clinically important difference thought these trends did not reach significance. Thus, there appears to be no deleterious consequences of local ZA administration on the biomechanical performance of the fusion mass at the 8-week time frame.

Author contributions

1. Ashley Strickland: Principal investigator throughout entire study, including design, performing experiments, carrying out evaluations, data analysis, and manuscript write-up.
2. Daniel Cavanaugh: Helped formulate study design, advising throughout study, and assisting with manuscript editing.
3. William Leatherwood: Assisted in evaluations-animal surgeries, carried out animal protocol, formulated manuscript and edits, corresponding author.
4. James Raynor: Assisted in carrying out evaluations and analyzing data.
5. Alexander Brown: Assisted in carrying out evaluations and analyzing data.
6. Paul Weinhold: Supervised all efforts throughout the study, from experimental design, performing experiments, carrying out evaluations, data analysis, and manuscript editing and preparation.

All authors have read and approved the final submitted manuscript.

Disclosures

The Authors declare that there is no conflict of interests.

Acknowledgements

Financial support for this project was provided by faculty development funds of Daniel Cavanaugh, MD for animal purchase, and The UNC Orthopaedic Research Fund for animal housing cost, imaging cost, and histology preparation.

Fig. 4. Representative images of toluidine blue stained specimens from each treatment group. There is evidence of bony remodeling in all groups, but the ZA200 specimens demonstrated areas of fibrous tissue formation around un-remodeled bony fragments (*).

References

- Hirsh BP, Unnanuntana A, Cunningham ME, Lane JM. The effect of therapies for osteoporosis on spine fusion: a systematic review. *Spine J.* 2013;13(2):190–199<https://doi.org/10.1016/j.spinee.2012.03.035>.
- von Knoch F, Jaquiere C, Kowalsky M, et al. Effects of bisphosphonates on proliferation and osteoblast differentiation of human bone marrow stromal cells. *Biomaterials.* 2005;26(34):6941–6949<https://doi.org/10.1016/j.biomaterials.2005.04.059>.
- Im GI, Qureshi SA, Kenney J, Rubash HE, Shanbhag AS. Osteoblast proliferation and maturation by bisphosphonates. *Biomaterials.* 2004;25(18):4105–4115<https://doi.org/10.1016/j.biomaterials.2003.11.024>.
- Giuliani N, Pedrazzoni M, Negri G, Passeri G, Impicciatore M, Girasole G. Bisphosphonates stimulate formation of osteoblast precursors and mineralized nodules in murine and human bone marrow cultures in vitro and promote early osteoblastogenesis in young and aged mice in vivo. *Bone.* 1998;22(5):455–461[https://doi.org/10.1016/S8756-3282\(98\)00033-7](https://doi.org/10.1016/S8756-3282(98)00033-7).
- Bobyn JD, Thompson R, Lim L, Pura JA, Bobyn K, Tanzer M. Local alendronic acid elution increases net periimplant bone formation: a micro-CT analysis. *Clin Orthop Relat Res.* 2014;472(2):687–694<https://doi.org/10.1007/s11999-013-3120-6>.
- Tanzer M, Karabasz D, Krygier J, Cohen R, Bobyn J, Dennis: the Otto Aufranc award: bone augmentation around and within porous implants by local bisphosphonate elution. *Clin Orthop Relat Res.* 2005;441:30–39<https://doi.org/10.1097/01.blo.0000194728.62996.2d>.
- Pura JA, Bobyn JD, Tanzer M. Implant-delivered alendronate causes a dose-dependent response on net bone formation around porous titanium implants in canines. *Clin Orthop Relat Res.* 2016;474(5):1224–1233<https://doi.org/10.1007/s11999-016-4714-6>.
- Yasen M, Li X, Jiang L, Yuan W, Che W, Dong J. Effect of zoledronic acid on spinal fusion outcomes in an ovariectomized rat model of osteoporosis. *J Orthop Res.* 2015;33(9):1297–12304<https://doi.org/10.1002/jor.22763>.
- Bransford R, Goergens E, Briody J, Amanat N, Cree A, Little D. Effect of zoledronic acid in an L6–L7 rabbit spine fusion model. *Eur Spine J.* 2007;16(4):557–562<http://doi.org/10.1007/s00586-006-0212-y>.
- Yuan W, James AW, Asatrian G, et al. NELL-1 based demineralized bone graft promotes rat spine fusion as compared to commercially available BMP-2 product. *J Orthop Sci.* 2013;18(4):646–657<http://doi.org/10.1007/s00776-013-0390-5>.
- Jakobson T, Baas J, Bechtold JE, Elmengaard B, Soballe K. The effect of soaking allograft in bisphosphonate: a pilot dose-response study. *Clin Orthop Relat Res.* 2010;468(3):867–874<http://doi.org/10.1007/s11999-009-1099-9>.
- Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res.* 2010;25(7):1468–1486<http://doi.org/10.1002/jbmr.141>.
- Willie BM, Birkhold AI, Razi H, et al. Diminished response to in vivo mechanical loading in trabecular and not cortical bone in adulthood of female C57Bl/6 mice coincides with a reduction in deformation to load. *Bone.* 2013;55(2):335–346<http://doi.org/10.1016/j.bone.2013.04.023>.
- Robinson ST, Svet MT, Kanim LA, Metzger MF. Four-point bending as a method for quantitatively evaluating spinal arthrodesis in a rat model. *Comp Med.* 2015;65(1):46–50<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4396928/>.
- Metzger MF, Kanim LEA, Zhao L, Robinson ST, Delamarter RB. The relationship between serum vitamin D levels and spinal fusion success: a quantitative analysis. *Spine.* 2015;40(8):E458–E468<https://doi.org/10.1097/BRS.0000000000000801>.