Improved Spinal Fusion Through Targeted Delivery of Abaloparatide

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INTRODUCTION

With over half a million spinal fusions performed in the United States every year, and with recovery time increasing with age, there is a need for alternative and affordable treatments. Extended postoperative immobility significantly impacts the risk for comorbidities and quality of life for patients. Current therapies rely on the application of a large amount of therapeutic at the time of surgery, which leads to side effects, such as ectopic mineralization and osteoclast-mediated bone resorption (1,2). We have developed a spinal fusion targeted peptide that can be delivered systemically without eliciting the skeletal side effects that plague existing therapeutics.

AIM

Existing strategies for spinal fusion require a surgery to apply the drug and only allow for one application. Application of therapeutics such as BMP2 suffer from leakage of the compound which causes bone to grow into the surrounding soft tissue, windpipe, and spinal cord. Our lab has developed a bone targeting system based on acidic oligopeptides that is more biocompatible and has a more relevant therapeutic half-life and that lacks the skeletal side effects that plague existing therapeutics. We had previously demonstrated this system to be effective at selectively delivering therapeutics to bone fractures from a systemic injection at a distal site. Here we demonstrate that the same targeting system could be used non-invasively to systemically administer therapeutics that chemically home to the spinal fusion sites.

METHOD

- In vitro activity of targeted Abalo was tested using cAMP stimulation activity in UMR106 cells employing a cAMPGlo assay kit (Promega)
- Binding constant of targeted Abalo to hydroxyappetite was estimated using Elisa employing hydroxyatite coated magnetic beads
- Once it was established that acidic oligopeptides could be targeted successfully to spinal fusion an efficacy study in both male and female skeletally mature Sprague Dawley rats(n=10/sex) was performed using a bilateral L4-L5 posterolateral lumbar spinal fusion with a 5x7.5-mm collagen sponge (RCM6 Ace Surgical supplies).
- 60 rats were randomly assigned to one of three treatments : (i) PBS (vehicle) control; (ii) 10µg BMP2; (iii) 38 nmol/kg twice a week of acidic oligopeptide targeted Abaloparatide (Abalo46-DGlu20).
- The initial dose of all drugs was administered on the sponge, and then vehicle control and Abalo46-DGlu20 (targeted abalo) were administered subcutaneously twice a week for 8 weeks.
- The spinal fusion mineralization was assessed using a Quantum GX µCT from PerkinElmer. Each rat was scanned weekly during weeks 3-8.
- The scans were blindly scored by 2 separate individuals.
- At the end, the lumbar was removed and were blindly manually palpated and submitted to mechanical evaluation via 3-point bend (Instron).
- All animal experiments were performed in accordance with protocols approved by Purdue University's Institutional Animal Care and Use Committee (IACUC).

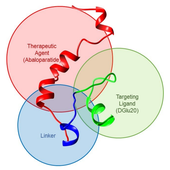

Fig.1. We synthesized fracture-targeted anabolic agents by synthesizing Abaloparatide factors (red) in tandem with a spacer (blue)) and a hydroxyapatite-binding acid oligopeptide (green).

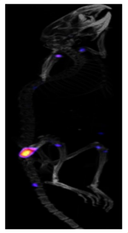

Fig.2.. Demonstration of fracture homing by D-Glu20 targeting (Tc-labeling)

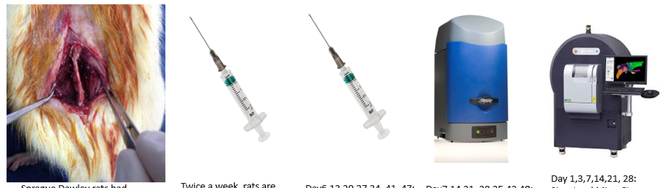

Fig. 3. Longitudinal imaging study design for spinal fusion

RESULTS

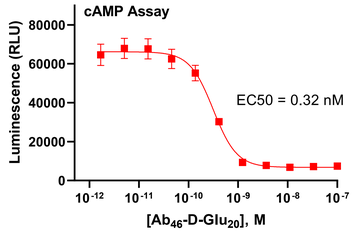

Fig.4. Targeted Abaloparatide was assessed in a cAMP-Glo assay on UMR106 cells that were treated with the drug for 1 hour. EC50 of targeted abalo was 0.32 nM which was similar to the value seen for ablo under similar conditions (0.48 nM).

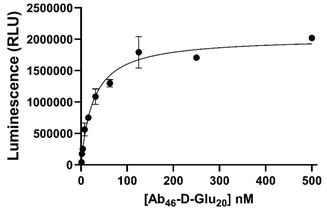

Fig.5. *In Vitro* Assessment of binding affinity of Acidic oligopeptide targeted abaloparatide to Hydroxyapatite. Even with the addition of linker and large abaloparatide payload, the acidic oligopeptides had a high affinity interaction with hydroxyapatite. The binding constant (K_D) was calculated to be 27 nM.

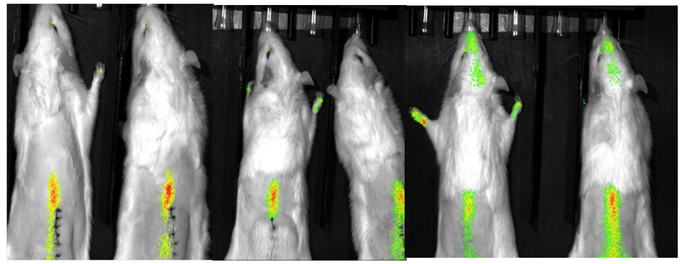

Fig.6..Biodistribution of targeted near-IR fluorescent molecule (S056) 24 hours post-injection in bilateral posterolateral lumbar spinal fusion (2 weeks post-surgery) in three different main scaffold types for spinal fusions. Localization primarily occurs in spine. Localization see on paws and head are due to grooming with paws contaminated by dye urinated out.

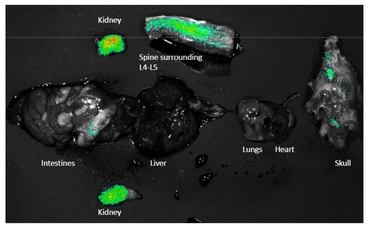

Fig.7. Localization of acidic oligopeptide targeted near inferred dye in rat with collagen scaffold 2 weeks after surgery and 24 hours post injection.

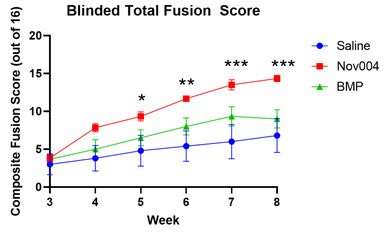

Fig. 8. *In vivo* fusion healing efficacy of targeted abalo on male Sprague Dawley rats (n=10) over 8 weeks after bilateral posterolateral lumbar fusion of L4-L5. analysis. Scores were assigned (0-4) on osteointegration, density, bridging of both bilateral spinal fusions in micro CT scans of the rats. Scores were assigned by two individuals blinded to the treatment reviewing randomized samples. Total score is sum of all 4 scores combined. Significance determined by One-way ANOVA.

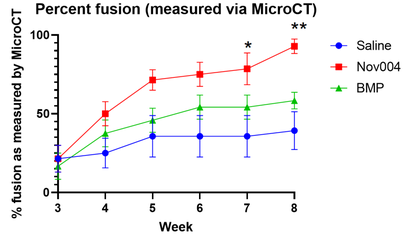

Fig.9. *In vivo* fusion healing efficacy of targeted abalo on male Sprague Dawley rats (n=10) over 8 weeks after bilateral posterolateral lumbar fusion of L4-L5. Fusion is assessed by completed osteointegration as seen by micro CT. This was assed in a randomized fashion by 2 individuals blinded to the treatment groups.

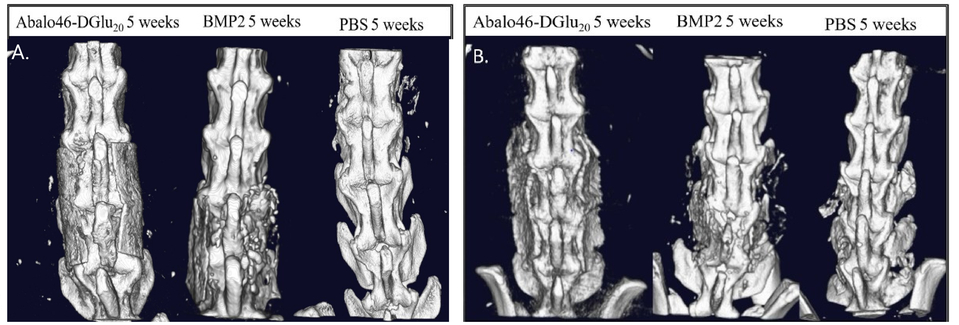

Fig.10. Spinal fusion in Sprague Dawley rats(n=10/sex) was performed using a 5x7.5-mm collagen sponge (RCM6 Ace Surgical supplies). 60 rats were randomly assigned to one of three treatments phosphate buffered saline vehicle control, 10µg BMP2, or 38nmol/kg twice a week of acidic oligopeptide targeted Abaloparatide (Abalo46-DGlu20). The initial dose of all drugs was administered on the sponge and then vehicle control and Abalo46-DGlu20 were administered subcutaneously twice a week. A) represents female rats and (B) are male rats.

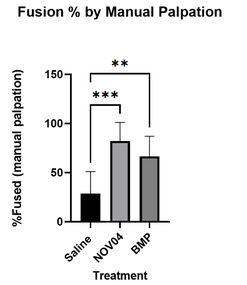

Fig. 11. male Sprague Dawley rats (n=10) after 8 weeks after bilateral posterolateral lumbar fusion of L4-L5. analysis. The lumbar region was excised postmortem and manually palpitated and evaluated for fusion between L4 and L5

CONCLUSIONS

- The targeted abaloparatide exhibited similar in vitro activity in UMR106 cells as the non-targeted drug.
- Even with the addition of linker and large abaloparatide payload, the acidic oligopeptides had a high affinity interaction with hydroxyapatite.
- The targeted fluorophores were able to localize selectively in the spinal fusion over the rest of the body in all three types of scaffolds evaluated from a subcutaneous injection at a distal site.
- The treatment group receiving Abalo46-DGlu20 had their scaffolds mineralize earlier than either the vehicle control or BMP2 treatment groups in both male and female rats with significant differences being observed as early as 4 weeks post-surgery. Abalo46-DGlu20 had more consistent success in fusion of the vertebrae than the other 2 treatments.
- There were no significant differences in total volume of the scaffolds but the density of the mineralized scaffold was greatest in the Abalo46-DGlu20 treated group.
- The potential earlier mineralization stimulated by Abalo46-DGlu20 seemed to be responsible for the improvement of the successful integration of mineralized scaffolds with the transverse processes and could play a role increasing the success rate of fusions in the future. The noninvasive method of this drugs administration should allow for the ease of its integration into current clinical practices.

REFERENCES

1. Martin BI, Mirza SK, Spina N, Spiker WR, Lawrence B, Brodke DS. Trends in Lumbar Fusion Procedure Rates and Associated Hospital Costs for Degenerative Spinal Diseases in the United States, 2004 to 2015. Spine (Phila. Pa. 1976). 2019;44(5):369–76. A
2. James AW, LaChaud G, Shen J, Asatrian G, Nguyen V, Zhang X, Ting K, Soo C. A Review of the Clinical Side Effects of Bone Morphogenetic Protein-2. Tissue Eng. - Part B Rev. 2016;22(4):284–97.
3. Hunter DJ. Spondylolysis and spondylolisthesis: prevalence and association with low back pain in the adult community-based population. 2013;34(2):199–205.

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