Targeted Delivery of GSK-3β Inhibitor Loaded Nanoparticles for Fracture Healing in a Murine Nonunion Model

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INTRODUCTION:

GSK-3β inhibitors, which activate the Wnt/β-catenin signaling pathway, are a promising option to accelerate fracture healing. However, poor biodistribution and off-target effects are a significant barrier. A novel bone targeted nanoparticle (BT-NP) delivery system has been developed that enhances bone biodistribution of these drugs and limiting off-target effects. While this system has demonstrated efficacy in expediting routine fracture healing, it has not been investigated in a nonunion setting. Our hypothesis is that treatment with BT-NP will increase fracture healing in a murine nonunion model.

METHODS:

An atrophic femur nonunion model was created in 14-16 week old mice using a tapered plate and a 3.0 mm mid-diaphyseal osteotomy. Peptides with a high affinity for tartrate-resistant acid phosphatase were conjugated to poly(styrene-alt-maleic anhydride)-b-poly(styrene) nanoparticles. The GSK-3β inhibitor (3-amino-6-(4-((4-methylpiperazin-1-yl)-sulfonyl)phenyl)-N-(pyridin-3-yl)pyrazine-2-carboxamide) was loaded into the nanoparticle cores, creating the fracture-targeting nanoparticle delivery system (BT-NP). Mice were randomized to three groups: early BT-NP (injected 3 days postoperatively), late BT-NP (injected 2 weeks postoperatively), and saline control. Femurs were harvested at 2, 4, 8, and 12 weeks postoperatively. Bone healing parameters were assessed using microCT and included bone volume, bone volume fraction, mineralization density, trabecular thickness, and trabecular number. A callus formation score from 0 to 7 was assigned based upon the degree of bony bridging present on microCT according to a previously described scoring system. Bone healing parameters at each timepoint were compared between the control group and either treatment group using independent t-tests. Differences in bone healing between timepoints were also assessed for each cohort using independent t-tests. Statistical significance was set at $p<0.05$.

RESULTS:

A total of 107 mice underwent a femoral nonunion procedure. Bone healing was assessed via microCT on 3-5 samples per group at 2-4 week timepoints and 6-9 samples per group at 8-12 week timepoints. The early BT-NP group demonstrated increased trabecular thickness at 8 weeks compared to controls (0.294mm vs. 0.259mm, $p=0.020$). No other differences in bone volume, bone volume fraction, mineralization density, trabecular thickness, trabecular number, or callus formation scores were identified between early BT-NP and controls or late BT-NP and controls at any timepoint (**Table 1**). Within each treatment group, there was no significant increase in bone formation identified between timepoints ($p<0.05$ for all). Additional histologic analyses are ongoing.

DISCUSSION AND CONCLUSION:

These results demonstrate the successful establishment of a murine nonunion model that does not develop bony healing for at least 12 weeks. This has implications for future nonunion investigations as previous murine models have not been as reliable over this length of time. Treatment with BT-NP did not lead to the robust fracture healing in the nonunion model previously observed in a conventional fracture model. This highlights the differences in biology between a nonunion and conventional fracture site. Further investigation is needed to better understand and optimize BT-NP delivery in a nonunion model. Finally, our results suggest that therapies demonstrating success in routine fractures may not necessarily translate to a nonunion setting. This is an important consideration for other bone healing applications to focus efforts on clinically relevant scenarios.

Table 1. microCT Bone Analysis

	Controls	Early Treatment	†p-value	Late Treatment	†p-value
2 Weeks	N=3	N=3			
Bone Volume (Mean ± SD, mm^3)	3.634± 0.283	3.800 ± 0.304	0.526		
Bone Volume Fraction (Mean ± SD)	0.063 ± 0.017	0.089 ± 0.004	0.065		
Mineralization Density (Mean ± SD, mgHA/ccm)	1355.4 ± 38.9	1361.6 ± 24.7	0.828		
Trabecular Thickness (Mean ± SD, mm)	0.279 ± 0.021	0.262 ± 0.019	0.351		
Trabecular Number (Mean ± SD, 1/mm)	0.893 ± 0.183	1.024 ± 0.134	0.374		
Callus Formation Score (Mean ± SD)	1.0 ± 0	1.0 ± 0	1.000		
4 Weeks	N=4	N=5		N=4	
Bone Volume (Mean ± SD, mm^3)	2.724 ± 0.137	3.424 ± 0.770	0.083	2.985 ± 0.407	0.512
Bone Volume Fraction (Mean ± SD)	0.067 ± 0.034	0.073± 0.027	0.762	0.102 ± 0.029	0.130
Mineralization Density (Mean ± SD, mgHA/ccm)	1320.6 ± 48.8	1323.8 ± 28.1	0.890	1293.3 ± 17.6	0.276
Trabecular Thickness (Mean ± SD, mm)	0.258 ± 0.013	0.255 ± 0.012	0.792	0.269 ± 0.014	0.260
Trabecular Number (Mean ± SD, 1/mm)	0.861 ± 0.324	0.871± 0.168	0.950	1.080 ± 0.202	0.216
Callus Formation Score (Mean ± SD)	1.0 ± 0	1.2 ± 0.4	0.317	1.0 ± 0	1.000
8 Weeks	N=8	N=7		N=8	
Bone Volume (Mean ± SD, mm^3)	3.360 ± 0.762	3.527 ± 0.727	0.665	3.108 ± 0.710	0.501
Bone Volume Fraction (Mean ± SD)	0.085 ± 0.035	0.078 ± 0.024	0.682	0.070 ± 0.028	0.323
Mineralization Density (Mean ± SD, mgHA/ccm)	1326.8 ± 41.0	1330.7 ± 22.8	0.804	1324.4 ± 22.7	0.877
Trabecular Thickness (Mean ± SD, mm)	0.259 ± 0.029	**0.294 ± 0.023**	**0.020**	0.278 ± 0.027	0.166
Trabecular Number (Mean ± SD, 1/mm)	0.948 ± 0.119	0.865 ± 0.120	0.315	0.832 ± 0.204	0.151
Callus Formation Score (Mean ± SD)	1.1 ± 0.4	1.1 ± 0.4	0.925	1.1 ± 0.4	1.000
12 Weeks	N=9	N=8		N=6	
Bone Volume (Mean ± SD, mm^3)	3.090 ± 0.754	3.246 ± 0.954	0.692	3.791 ± 0.603	0.111
Bone Volume Fraction (Mean ± SD)	0.070 ± 0.020	0.071 ± 0.025	0.971	0.086 ± 0.023	0.224
Mineralization Density (Mean ± SD, mgHA/ccm)	1334.6 ± 17.2	1326.1 ± 54.9	0.650	1355.1 ± 34.2	0.322
Trabecular Thickness (Mean ± SD, mm)	0.273 ± 0.027	0.273 ± 0.047	0.986	0.294 ± 0.029	0.292
Trabecular Number (Mean ± SD, 1/mm)	0.773 ± 0.125	0.875 ± 0.150	0.189	0.901 ± 0.199	0.132
Callus Formation Score (Mean ± SD)	1.0 ± 0	1.75 ± 1.4	0.141	1.8 ± 1.2	0.133

SD = Standard deviation

***Boldface** indicates statistical significance.

†p-values calculated using independent t-tests