Fracture-Targeted Anabolic Therapy of Osteogenesis Imperfecta

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Background

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a genetic disorder affecting ~1 in 15,000 births. Phenotypes range from occasional bone fractures due to mild trauma to severe skeletal deformities and extremely fragile bones. Treatment of many OI fractures differ little from traditional fracture therapy and rely primarily on stabilization. Some effort to augment the repair process in OI patients with drugs, such as teriparatide, has yielded only mild improvements, potentially due to the insufficient concentrations at fracture sites.

We have developed a systemically administered fracture-targeted therapeutic with a high affinity to bone fractures. By improving the specificity of anabolics to fractures, we see significantly accelerated bone repair, reduced systemic affects, and no ectopic bone formation. We have previously demonstrated excellent fracture healing compared to saline, teriparatide, and abaloparatide in healthy, osteoporotic, and diabetic mice. Here we explore efficacy in OI fractures.

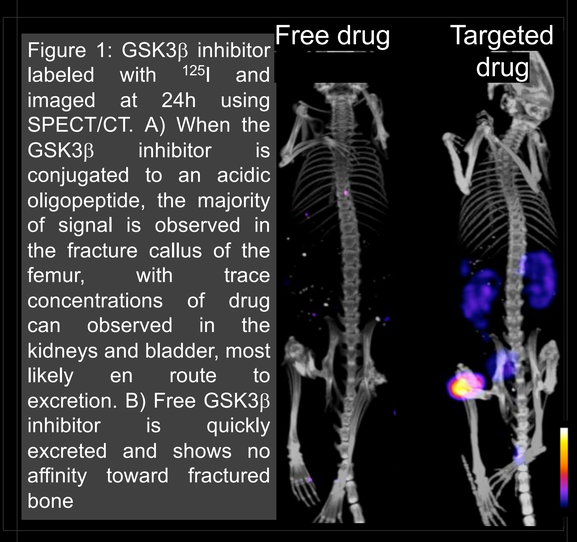


Figure 1: GSK3β inhibitor labeled with ^{125}I and imaged at 24h using SPECT/CT. A) When the GSK3β inhibitor is conjugated to an acidic oligopeptide, the majority of signal is observed in the fracture callus of the femur, with trace concentrations of drug can observed in the kidneys and bladder, most likely en route to excretion. B) Free GSK3β inhibitor is quickly excreted and shows no affinity toward fractured bone

Methods

Our fracture-targeted bone anabolic agent was prepared by synthesizing an abaloparatide-like peptide conjugated to a hydroxyapatite-homing acidic oligopeptide (named Ab_{46}-D-Glu_{20}).

In vivo experiments were conducted in heterozygous (-/+) and homozygous (-/-) *Col1a2^{oim}* mice representing a mild type I OI and severe type III OI respectively. Femurs were stabilized and fractures induced with an Einhorn 3-point bending device. Mice were dosed with 38 nmol/kg/d of Ab_{46}-D-Glu_{20} or saline. Following a 4-week study in heterozygous mice and 6-week in homozygous mice, fracture callus densities were measured using microCT. A marked increase in bone volume fraction (>85%) was observed in each Ab_{46}-D-Glu_{20} group over the saline groups. Moreover, mechanical testing yielded between 220% and 300% increase in force to fracture in the Ab_{46}-D-Glu_{20} over the saline control groups.

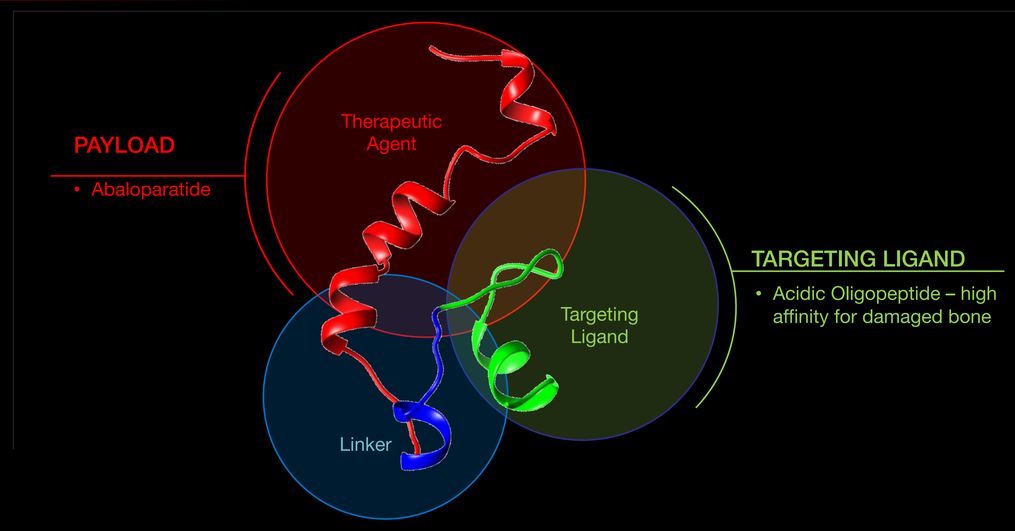
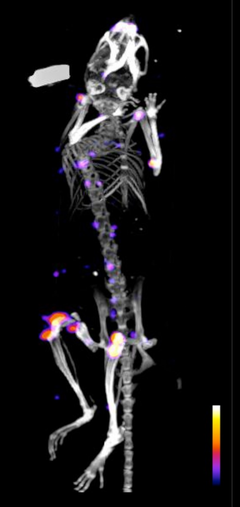


Figure 2: We synthesized a fracture targeted anabolic agent by synthesizing abaloparatide (yellow) in tandem with a spacer (blue) and a hydroxyapatite binding acid oligopeptide (magenta). This combination was selected for several reasons:

- Abaloparatide demonstrated similar effects as teriparatide in osteoporosis clinical trials
- Works in a paracrine/autocrine manner, whereas Forteo functions in an endocrine process
- Simple administration (Subcutaneous dosing)

Figure 5: Image of the colocalization of targeted drug overlayed with a CT of a type III OI mouse. The targeted drug was labeled with $Tc^{99}m$ and injected via tail vein into a type III OI mouse. A significant amount of accumulation was observed in the left femur which had a 4 week femoral fracture. Unlike a otherwise healthy mouse, various other areas of accumulation are also observed when targeting OI.



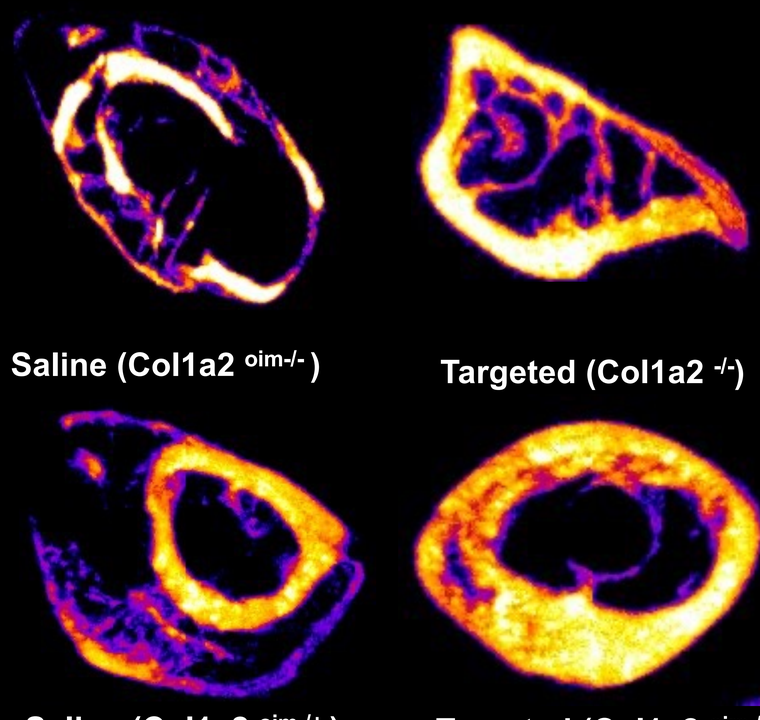
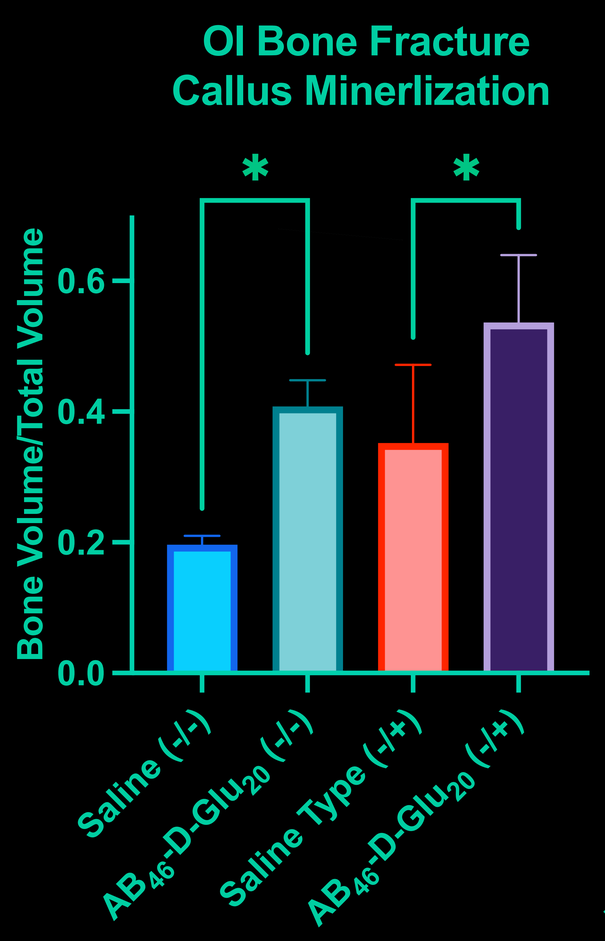
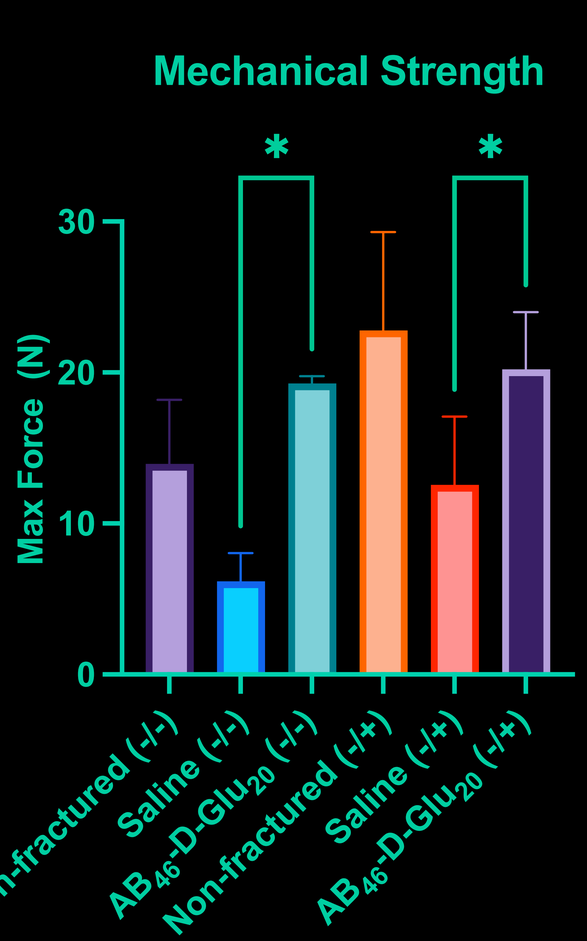


Figure 3 (left): Median microCT images from fracture cross sections in heterozygous (-/+) and homozygous (-/-) mouse femurs in Figure 4. Each image is a composite of 20 microCT slices. Yellow and orange colors indicate higher bone densities than purple and blue. In general, the white/light yellow areas constitute original cortical bone and the cooler colors correspond to new trabecular and woven bone. The Ab_{46}-D-Glu_{20} outperforms both the saline control.

Figure 4 (below): Morphometric and mechanical analysis of female heterozygous (-/+) and homozygous (-/-) fractured bones 6-weeks post fracture. Mice were dosed twice a week subcutaneously.





Discussion

In a practical sense, when a physician observes radiographic healing, the patient is much more likely to be cleared to function without a cast or splint and to continue with normal activities. In the process of measuring bone volume fraction, we limited the measurement to the bridging volume of the callus in order to focus our measurements on radiographic healing, and we were able to show significant improvement of this measure with administration of our targeted anabolic agent over the control. However, as with most OI cases, the *Col1a2^{oim}* mouse model causes a defect in collagen formation, potentially leading to poor bone quality, and it is plausible that even with demonstrable radiographic healing that the overall mechanical quality of bone may still be impaired. Whereas bone volume fraction is an excellent measure of radiographic healing, mechanical testing elucidates the quality of the bone. In this quantitative measure, we saw the greatest improvement in fracture healing with a dramatically higher force required to refracture the bone. Implications for this marked improvement in mechanical stability in the context of OI could be profound. Where in a healthy patient, a physician removes a cast when bridging is complete, OI patients often have have casts removed earlier to prevent non-use bone loss. By speeding fracture repair in OI patients, it is plausible that one could heal a fracture before the patient has to have stabilization removed, reducing the risk of refracture.

Conclusions

OI is an underserved population that would greatly benefit from a treatment that, in conjunction with conventional therapy, not only improves fracture repair but is safe enough to use several times throughout their lives. By targeting bone anabolic agents to bone fractures, we can deliver sufficient concentrations of anabolic agent to the fracture site to safely accelerate healing.

Acknowledgments

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