Engineering Abaloparatide to Accumulate Locally in Fracture Calluses Following Systemic Administration

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PURPOSE: Traditional fracture repair has been approached primarily from a mechanical engineering strategy. Namely, the objective is to only immobilize the fracture and to ignore biological stimulation. This results in 300,000 to 700,000 non-union and delayed-union bone fractures in the United States each year, causing significant morbidity, mortality, and lost productivity. In general, conventional therapy has focused on mechanically stabilizing fractures with little consideration of a pharmaceutical approach to accelerate fracture repair.[1]Efforts to biologically stimulate bone have focused on locally-applied bone anabolic agents (such as BMP-2) have demonstrated improved fracture repair but are limited to single administration during an invasive surgery. In contrast, systemically-administered anabolic agents such as Abaloparatide and Teriparatide are safe and can be dosed multiple times but fail to achieve therapeutic concentrations at the fracture site.

We have developed a novel solution to this problem: namely, a fracture-targeted bone anabolic agent that is both potent and shows no toxicities near the therapeutic range. Following systemic injection, the targeted bone anabolic accumulates selectively on a bone fracture surface and improves the rate of fracture repair as well as the quality of the bone. By selectively targeting the fracture itself, we avoid the invasive surgery and ectopic bone growth associated with application of local anabolic agents. The systemic administration also provides opportunity to deliver multiple doses, sustaining the stimulation during specified stages of the healing process.

METHODS

To achieve the above objective, we synthesized an engineered fragment of parathyroid hormone related protein fragment 1-46 with substitutions at $Glu^{22,25}$, $Leu^{23,28,31}$, Aib^{29}, $Lys^{26,30}$ (ePTHrP). On the C-terminus of the ePTHrP we conjugated a bone mineral-(hydroxyapatite-) targeting acidic oligopeptide forming targeted-ePTHrP. The negatively charged oligopeptide gives targeted-ePTHrP a remarkable specificity and homing ability towards raw hydroxyapatite and exponentially improves accumulation of the conjugate in fracture sites, bone graft sites, or where bone is surgically cut. With fracture specific accumulation achieved the attached ePTHrP reaches sufficient concentrations to dramatically accelerate healing.

Murine *in vivo* experiments were conducted on female Swiss Webster mice (15 per group). Femoral fractures were induced with an Einhorn 3-point bending device (RISystems) and stabilized by locking nail. Mice were dosed daily, every other day, every third day, or weekly with with 0.03, 0.3, 3 nmol/kg/dose of targeted-ePTHrP, non-conjugated (free) ePTHrP, or saline. Following a 3-week study, fracture callus densities were measured using microCT followed by mechanical testing.

Canine *in vivo* experiments were conducted on 1-year-old male beagles. Beagles underwent a 10 mm bilateral ulnar ostectomy. Two dogs in the treatment groups and Three dogs in the control group were dosed daily with either targeted-ePTHrP 0.5 nmol/kg/d, 5 nmol/kg/d, or saline respectively. Dogs were x-rayed weekly for the first 6 weeks and then every other week thereafter. Blood samples were collected and analyzed throughout the study for aberrations in serum chemistry levels and cellular levels. Following the study, heart, lung, liver, spleen, kidney, bladder, and injection site samples were collected and analyzed by a veterinary pathologist.

One tailed ANOVA followed by Dunnett's post-hoc test was used to establish significance. All animal experiments were conducted as described in approved IACUC protocols. $P<0.05$ was considered significant.

RESULTS SECTION:
In the murine studies we observed a marked increase in fracture callus size and an increase in bone deposition compared to saline was observed in the all daily dosed targeted-ePTHrP groups. When dosed every other day or every third day, the significant improvements were observed in doses equal to or greater than 0.3nmol/kg/dose. Once a week dosing required 3 nmol/kg/dose in order to significantly improve fracture repair. Most impressive was the degree to which fracture repair was improved. Comparing daily 0.1 nmol/kg/dose and saline, bone deposition and max load doubled and the energy the required to refracture the bones increased nearly 9-fold.

In the canine studies, we observe a seven -old improvement in closure of the ostectomy gap of 0.5 nmol/kg/d compared to saline controls ($P<0.05$). In addition, no significant differences in weight, blood chemistry levels or organ histology were observed in the (higher dose) 5 nmol/kg/d treatment vs. saline controls. Slightly higher alkaline phosphatase levels were observed in the treated groups but were not significant.

CONCLUSIONS:
Although clinical trials have been performed on systemically administered bone anabolic agents for fracture repair (i.e. teriparatide), to date, no such anabolics have been approved for this use. These trials demonstrated an evidence that anabolic activity was occurring at the fracture site, but were not sufficient to meet FDA required end-points.[2] It is likely that if sufficient drug were to accumulate at a fracture site then accelerated fracture repair would be possible. Previously, we have demonstrated that anabolic-conjugated acidic oligopeptides greatly improve fracture specific accumulation.[3] Here, by engineering a safe, clinically proven, parathyroid hormone receptor agonist with an acidic oligopeptide accelerated fracture repair and improved mechanical properties in mice. Furthermore, in canine critical sized defect ostectomies, improved ostectomy closure was observed. More importantly, we observed no toxicities at 10-fold the therapeutic dose.

Technology advancement in fracture repair has been slowed by the limiting focus to mechanical stability rather than improving the biological environment. By creating a systemically administered drug that locally accumulates at the fracture site, we are able to deliver sufficient concentrations of anabolic agent to the fracture site to accelerate healing.

REFERENCES: Include references here. (References are Optional)
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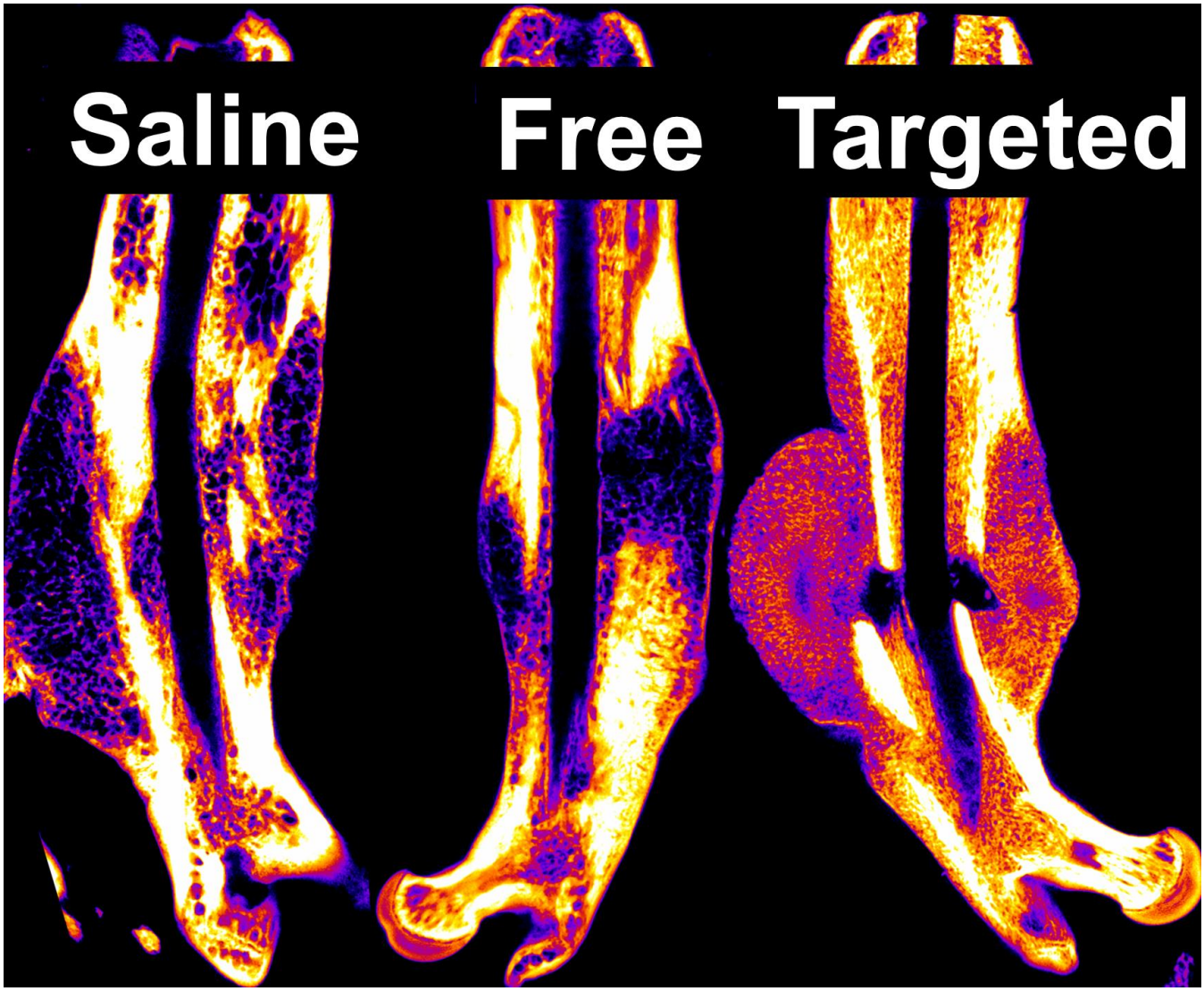

Representative microCT images of mouse femurs following the 3-week study.