Fracture Targeted Parathyroid Hormone Agonist As An Effective Pharmaceutical For Bone Repair in Murine and Canine Models

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Disclosures:

Bone fractures result in significant morbidity, mortality, and lost productivity worldwide. Little has been done to improve fractures with pharmaceuticals. Current therapeutics have limitations due to invasive administration routes, side effects, or inefficacy. We have developed a potent, nontoxic fracture-targeted bone anabolic agent that, following systemic injection, accumulates selectively on a bone fracture surface and accelerates the rate of fracture repair. The targeted therapy avoids invasive surgery and improves fracture repair rate and quality without promoting ectopic bone growth or altering healthy nontargeted bones. It also allows for repeated administration.
Our targeted anabolic agent is constructed of a bone mineral hydroxyapatite-targeting oligopeptide conjugated to the non-signaling end of an engineered parathyroid hormone-related protein fragment 1-46 with substitutions at $Glu^{22,25}$, $Leu^{23,28,31}$, Aib^{29}, and $Lys^{26,30}$ (ePTHrP). The negatively-charged oligopeptide has been shown to target raw hydroxyapatite with remarkable specificity, while the attached PTHrP induces sustained and accelerated bone growth. We have repeatedly demonstrated the ability of this conjugate to accelerate bone fracture repair in multiple animal models of bone healing. In adult murine midshaft femoral fracture model (n=15), we observed a significant increase in fracture callus size, doubling in callus bone density, and a two-fold increase in callus bone deposition. After three weeks of treatment in the targeted-ePTHrP group over the saline group ($P<0.01$), we observed an increase in maximum force withstood and a six-fold increase in work-to-fracture. In a bilateral ulnar ostectomy (1cm) in adult beagle studies, we observed a closure of the ostectomy gap seven times as fast as saline controls ($P<0.05$). Additionally, no significant differences in weight or in a histological evaluation of the organs were observed in the treatment vs. saline controls. Fracture-targeted parathyroid hormone agonist is an effective pharmaceutical for bone repair in both murine and canine models and offers a promising potential therapeutic.

Although attempts have been made in developing a systemically administered fracture therapeutic for fracture repair, i.e. teriparatide, to date, no such anabolics have been approved for this use. In these studies there is evidence that anabolic activity was occurring at the fracture site, but at a level that did not meet FDA required end-points.[2] It is plausible that if sufficient drug were to be delivered to a fracture site then improved fracture repair would be possible. In previous studies, we demonstrated fracture specific accumulation bone anabolics can be achieved by modifying the drug with acidic oligopeptides.[3] Here, by modifying a safe, clinically proven, parathyroid hormone receptor agonist with an acidic oligopeptide we observe improved bone deposition and strength in mice. Furthermore, when administered to canine critical sized defect ostectomies, a more relevant and difficult model, we observe improved ostectomy closure.

OBJECTIVE:
The primary objective of this study was to evaluate the performance of a bone fracture targeted systemically administrable bone anabolic as a potential therapeutic for bone fracture repair. Currently all bone fracture repair therapeutic require local administration during surgery. However, the population that need the most assistance in repair bone fractures are not eligible for surgery. So, it was our goal to design an inject-able therapeutic to assist in bone fracture repair to reduce the invasiveness. The injectable nature of it allows for repair administration of the bone anabolic and for therapeutic effect throughout the entire bone fracture healing process. Targeting it to the bone fracture site reduces the toxicity and increases the efficacy.

METHODS

To achieve the above objective, a bone mineral-(hydroxyapatite-) targeting oligopeptide was conjugated to the non-signaling end of an engineered parathyroid hormone related protein fragment 1-46 with substitutions at $Glu^{22,25}$, $Leu^{23,28,31}$, Aib^{29}, $Lys^{26,30}$ (ePTHrP). The negatively charged oligopeptide has been shown to target raw hydroxyapatite with remarkable specificity, while the attached PTHrP has been demonstrated to induce sustained and accelerated bone growth under control of endogenous morphogenic regulatory factors. The conjugate's specificity arises from the fact that raw hydroxyapatite is only exposed whenever a bone is fractured, surgically cut, grafted, or induced to undergo accelerated remodeling. The hydroxyapatite-targeted conjugate can therefore be administered systemically (i.e. without invasive surgery or localized injection) and still accumulate on the exposed hydroxyapatite at the fracture site where it accelerates the healing process

Murine *in vivo* experiments were conducted on female Swiss Webster mice (10 per group). Femoral fractures were induced with a 3-point bending device and stabilized. Mice were dosed with 3 nmol/kg/d of targeted-ePTHrP, non-conjugated (free) ePTHrP, or saline. Following a 4-week study, fracture callus densities were measured using microCT.

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 group and Three dogs in the control group were dosed daily with either targeted-ePTHrP 0.5nmol/kg/d or saline respectively. Dogs were x-rayed weekly for the first 6 weeks and then every other week thereafter.

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 a 2-fold increase in bone deposition was observed in the targeted-ePTHrP group over the saline group ($P<0.01$). A significant doubling in bone density was also observed. Targeted-ePTHrP group fractured femurs were able to achieve their pre-fracture strength as early as 3 weeks compared to 9 weeks in the saline mice representing a 66% reduction in healing time.

In the canine studies, we observe a significantly higher closure of the ostectomy gap than saline controls ($P<0.05$). In addition, no significant differences in weight are observed in the treatment vs. saline controls. No significant difference between the control group and treatment groups was found in a histological investigation of the organs.

DISCUSSION:
Although attempts have been made in developing a systemically administered fracture therapeutic for fracture repair, i.e. teriparatide, to date, no such anabolics have been approved for this use. In these studies there is evidence that anabolic activity was occurring at the fracture site, but at a level that did not meet FDA required end-points.[2] It is plausible that if sufficient drug were to be delivered to a fracture site then improved fracture repair would be possible. In previous studies, we demonstrated fracture specific accumulation bone anabolics can be achieved by modifying the drug with acidic oligopeptides.[3] Here, by modifying a safe, clinically proven, parathyroid hormone receptor agonist with an acidic oligopeptide we observe improved bone deposition and strength in mice. Furthermore, when administered to canine critical sized defect ostectomies, a more relevant and difficult model, we observe improved ostectomy closure.

CLINICAL RELEVANCE::
The ability to accelerate bone fracture repair is a fundamental need that has not been addressed by conventional methods. By targeting bone anabolic agents to bone fractures, we can deliver sufficient concentrations of anabolic agent to the fracture site to accelerate healing, thus avoiding surgery and any ectopic bone growth associated with locally-applied bone anabolic agents.

REFERENCES: Include references here. (References are Optional)

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