Bone-Targeting Parathyroid Hormone Analogues Outperform Unmodified PTH in the Anabolic Treatment of Osteoporosis in Rats

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ABSTRACT SUMMARY

Novel bone-targeting Parathyroid Hormone-polyethylene glycol-Bisphosphonate (PTH-PEG-BP) conjugates were synthesized, characterized and evaluated for bioactivity, bone affinity and anabolic efficacy in vitro/vivo. Simple bisphosphonate (BP) moieties were conjugated to PTH using heterobifunctional PEG linkers in order to impart bone specificity for PTH after administration in a rat model of Osteoporosis, secondary to ovariectomy. Results for bone volume and new bone formation by Micro-CT, electron probe micro-analysis, and histological analysis showed significantly improved anabolic bone formation on treatment of osteoporotic rats using bone-targeting PTH-PEG-BP compared to currently marketed unmodified PTH.

INTRODUCTION

Teriparatide (recombinant PTH 1-34; marketed as FORTEO®, Eli Lilly) was approved by the US Food and Drug Administration (FDA) in 2002 for treatment of osteoporosis and has proven effective as an anabolic therapy. However, due to the rapid enzymatic degradation of PTH, treatment efficacy is restricted by the short half-life (2-3 min); in addition, PTH receptors exist in non-skeletal tissues, such as the kidneys and central nervous system, thereby negatively impacting bioavailability of the peptide hormone to bone cells.

In this study, we synthesized PTH-PEG-BP analogues with significantly enhanced bone targeting affinity, whilst retaining full PTH bioactivity on triggering PTH receptors, as shown in cell culture. Micro-CT based quantitative evaluation concluded higher therapeutic efficacy of PTH-PEG-BP in the treatment of a rat model of osteoporosis.

EXPERIMENTAL METHODS

PTH-PEG-BP analogues were prepared using “2-step” organic synthesis by reacting with MAL-PEG-NHS then Thiol-BP. Bioactivity was confirmed by cAMP generation after incubation with UMR-106 cells which constitutively express the PTH receptor. In-vitro bone binding affinity was assessed in an hydroxyapatite (HA) binding study. Sixteen Sprague-Dawley rats were ovariectomized (OVX) and remained untreated for 8 weeks to allow for the development of osteopenia, at which point they were dosed (in groups of 4) with identical 14.6 nmol/kg/day dosages of either unmodified PTH or PTH-PEG-BP, as previously published, and compared to 4 sham-operated controls. A proven sub-therapeutic dose of strontium ranelate (225 mg/kg/day) was orally administered to all rats over the final 8 weeks of the study in order to serve as a dynamic molecular tracer of new bone formation, as we have previously shown. In-vivo micro-CT scans of the proximal tibial metaphysis were performed at the 0, 4, 8, 12 and 16 week time-points, for quantitative analysis of trabecular bone volume and mineral density for each treatment regimen. Electron probe micro-analysis (EPMA) was used to identify elemental strontium in acetone-fixed tibial bone samples embedded in epoxy resin, and thin sections stained with H&E and Tetrachrome, for analysis of bone turnover.

RESULTS AND DISCUSSION

PTH-PEG-BP analogues were synthesized successfully (Figure 1). Both native PTH and PTH-PEG-BP triggered cAMP after incubation with UMR-106 cells which contain the PTH receptor. In-vitro HA binding assays indicated that up to 41% of PTH-PEG-BP was bound to
the mineralized HA pellet, compared to <10% for unmodified PTH.

Both unmodified PTH and bone-targeting PTH-PEG-BP showed a dramatic reversal in osteopenic bone volume changes upon administration, as measured temporally by *in-vivo* micro-CT. The initial significant loss of percent trabecular bone volume to tissue volume (%BV/TV) in all ovariectomized rats was abruptly halted following PTH therapy, with significant gains in new bone formation quantified 4 and 8 weeks after the introduction of PTH (Figure 2). Of particular note, daily PTH-PEG-BP significantly increased bone mass at a greater rate than that of unmodified PTH alone. Even once-weekly administration of PTH-PEG-BP was capable of a measurable anabolic response suggesting the potential for less frequent patient injection with further dosage refinement.

In contrast, OXV-untreated rats showed significantly reduced trabecular %BV/TV as a result of excessive bone resorption that did not return to positive bone balance for the entire 16 wk duration. Dynamic labeling of mineralizing bone surfaces under each treatment regimen was evidenced by detecting incorporated elemental strontium and confirmed the anabolic increase in newly formed bone with bone-targeting PTH-PEG-BP analogues (Figure 3).

**CONCLUSION**

Ours is the first report of a bone-targeting anabolic bone therapeutic. Compared to currently marketed unmodified PTH, these analogues showed significant affinity for bone mineral and significantly improved efficacy in terms of increasing bone volume and BMD in rats developing an osteoporosis-like condition. Bisphosphonate-mediated targeting of PEGylated PTH to bone represents a new class of targeted anabolic compound that has not previously been attempted.

**REFERENCES**


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