Effects of a New Conjugate Drug in a Rat Model of Postmenopausal Osteoporosis

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Abstract

Current treatments for osteoporosis generally fall into one of two categories: 1) anti-resorptive drugs that inhibit resorption, such as bisphosphonates (e.g. alendronate), and 2) anabolic drugs that promote bone formation, such as parathyroid hormone (PTH)\(^\text{\textdagger}\). However, inhibiting resorption eventually also suppresses formation, and long-term use of PTH has been associated with osteosarcoma in rats\(^\text{\textdagger}\). Better approaches are thus needed.

Prostaglandin E\(_2\) (PGE\(_2\)) is a locally acting fatty acid derivative that has bone-anabolic effects in vivo\(^\text{\textdagger\dagger}\), but its use is hampered by systemic side effects\(^\text{\textdagger\dagger}\). PGE\(_2\) acts on bone via the EP4 receptor on osteoblasts\(^\text{\textdagger\dagger}\), and synthetic EP4 receptor agonists have also been shown to promote bone formation in vivo, although systemic administration of such agonists still results in unwanted side effects\(^\text{\textdagger\dagger\dagger}\).

Exploiting the bone-binding property of bisphosphonates, our approach delivers the EP4 agonist (PGE\(_2\)) directly to bone sites by linking it with alendronate (ALN)\(^\text{\textdagger}1\). This study investigates the in vivo effects of the ALN-PGE\(_4\) conjugate using the osteovarietomized (OVX) rat model of postmenopausal osteoporosis.

**Conjugate Mechanism of Action:**

Figure 1. Mechanism of action of ALN-PGE\(_4\) conjugate. Step (1): Formyl conjugate ALN-PGE4 and selectively activates the ALN-binding domain of EP4 on osteoblasts. Step (2): ALN binds to bone matrix and delivers PGE4 to bone site. Local hydrolysis occurs in the bone environment cleaving the conjugate to form ALN-PGE4. Step (3): PGE4 binds to and stimulates bone formation, while ALN remains bound to bone site of action and thus decreases the bisphosphonate should also be observed via slow-release and/or more stable analogs of PGE2.

**Results**

**Effects on Trabecular Bone**

- CH resulted in increased osteoid volume, but percent osteoid volume was unchanged due to higher total bone volume (Fig 4 left panel).
- CL led to elevated bone formation relative to OV, comparable to or exceeding PG levels. EA resulted in decreased bone formation (Fig 4 right panel).
- Conjugate treatment led to dose-dependent increase in trabecular bone volume, formation of new trabeculae, but trabecular thickness was not increased (Fig 5).
- Vertebral trabecular BMD was dose-dependently increased due to conjugate treatment, but this did not translate into increased material strength (ultimate stress) despite greater load-bearing (ultimate load) compression testing (Fig 6).

**Effects on Cortical Bone**

- Conjugate treatment led to dose-dependent periosteal and endocortical bone formation and increased cortical porosity, which led to reduced BMD in the CH group (Fig 7 & 8 left panel). Load-bearing (ultimate load) was improved in CH due to large size, but material strength (ultimate stress) is decreased (Fig 8 right panel).

**Discussion**

Conjugate treatment increased cortical bone turnover in a dose-dependent manner, with periosteal and endocortical bone formation and elevated cortical porosity. This increased the load-bearing ability but compromised the material strength of diaphyseal cortical bone in the high dose group.

**Methods**

- In this curative experiment, three-month-old female Sprague-Dawley rats were ovariectomized, allowed to lose bone for 6 weeks, then treated for 6 weeks.
- Calsecium green was injected as a fluorescent marker for bone formation at 12 and 4 days before sacrifice.
- Treatment effects on tissue-level remodeling, bone density, and bone strength were evaluated.

Table 1. Animal treatment groups. ST and OV are negative controls. PG is positive control (etidronate), and ALN controls for conjugation. Saline was used as a vehicle for injection. *For histological analysis, CH group was given 15 mg/kg for 4 weeks. **For in vivo bone strength, dose range was reduced to 15 mg/kg at weekly intervals in weeks 2, 4, 6 due to side effects.

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**References**