Institute of Biomaterials & Biomedical Engineering
UNIVERSITY OF TORONTO

CIHR IRSC
Canadian Institutes of Health Research

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

Careesa C. Liu[1], Robert N. Young[2], and Marc Grynpas[1,3]

[1]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada;* [2]*Department of Chemistry, Simon Fraser University, Canada;* [3]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Canada*

INTRODUCTION

Current treatments for osteoporosis generally fall into one of two categories: 1) anti-resorptive drugs that inhibit resorption, such as bisphosphonates[1] (e.g. alendronate), and 2) anabolic drugs that promote bone formation, such as parathyroid hormone (PTH)[2]. However, inhibiting resorption eventually also suppresses formation, and long-term use of PTH has been associated with osteosarcoma in rats[3]. Better approaches are thus needed.

Prostaglandin E_2 (PGE_2) is a locally acting fatty acid derivative that has bone-anabolic effects in vivo[4-10], but its use is hampered by systemic side effects[10]. PGE_2 acts on bone via the EP4 receptor on osteoblasts[10,11], 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[10,12-14].

Exploiting the bone-binding property of bisphosphonates, our approach delivers the EP4 agonist (EP4a) directly to bone sites by linking it with alendronate (ALN)[15]. This study investigates the in vivo effects of the ALN-EP4a conjugate using the ovariectomized (OVX) rat model of postmenopausal osteoporosis.

Conjugate Mechanism of Action:

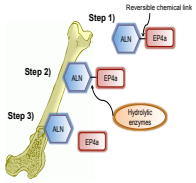
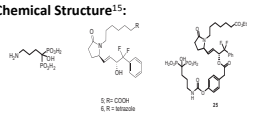


Fig 1. Mechanism of action of ALN-EP4a conjugate. *Step 1):* Reversibly conjugate ALN with EP4a and systemically administer the compound. *Step 2):* ALN binds to bone mineral and "delivers" EP4a to bone site. Local hydrolytic enzymes in the bone environment cleave the conjugation link to liberate EP4a. *Step 3):* EP4a is freed to stimulate bone formation, while ALN remains bound to bone.

Chemical Structure[15]:



ALN EP4a ALN-EP4a

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.
- Calcein green was injected as a fluorescent marker for bone formation at 12 and 2 days before sacrifice.
- Treatment effects on tissue-level remodeling, bone density, and bone strength were evaluated.

Table 1. Animal treatment groups. SV and OV are negative controls, PG is positive control for bone formation, and EA is control for conjugation. SQ=subcutaneous injection; IV=intravenous injection. *CH group was given 25 mg/kg injection in week 1 of treatment, but dosage was reduced to 15 mg/kg at biweekly intervals in weeks 2, 4, 6 due to side effects.

Group	n	Treatment	Route	Frequency	Total Dose/ Week
Sham Operated (SV)	11	Vehicle	SQ	Daily	0
Ovariectomized (OV)	12	Vehicle	IV	Weekly	0
Conjugate, Low Dose (CL)	12	5 mg/kg	IV	Weekly	5 mg/kg
Conjugate, High Dose (CH)	9	15 mg/kg	IV	Biweekly*	15 mg/kg
Separate EP4a + ALN (EA)	12	2.5 mg/kg each	IV	Weekly	5 mg/kg
Prostaglandin E2 (PG)	12	4 mg/kg	SQ	Daily	20 mg/kg

RESULTS

Overall Effects

- High dose (CH) led to significantly larger bones in length and cross-sectional area (Fig 2).
- Conjugate treatment resulted in dose-dependent increase in trabecular bone formation and cortical porosity (Fig 3).

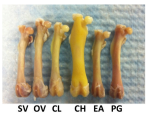


Fig 2. Photograph of right femurs.

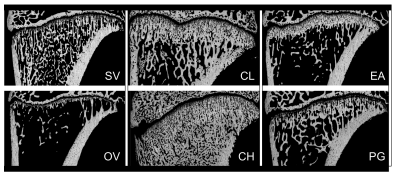


Fig 3. Back-scattered electron microscopy of proximal tibia (original magnification 100X). OVX resulted in significant trabecular bone loss compared to SV. CL was comparable to PG in trabecular bone formation, but CH was excessive.

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 with formation of new trabeculae, but trabecular thickness was not increased (Fig 5).

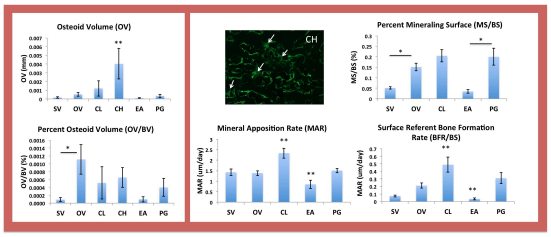


Fig 4. Undecalcified histomorphometry of the proximal tibial metaphysis at 100X magnification. *Left:* Goldner's trichrome stained 5μm sections. OV shows increased percent osteoid volume compared to SV. *Right:* Fluorescent markers on 7μm unstained sections. CH group was excluded from analysis due to diffuse labeling (white arrows) that prevented quantification. OVX resulted in increased MS/BS, confirming elevated turnover. *p<0.05 as indicated, **p<0.05 compared to each of the other groups. Mean ± SD.

- 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) under compression testing (Fig 6).

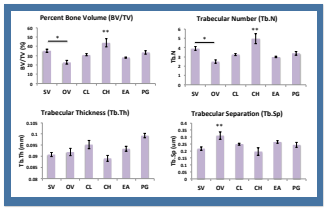


Fig 5. Micro-computed tomography (microCT) of secondary spongiosa in 6th lumbar vertebrae scanned at 11.6μm voxel size using the Skyscan 1174 system. OV is decreased in bone volume and trabecular number relative to SV, resulting in increased trabecular separation. Conjugate treatment led to dose-dependent increase in bone volume and trabecular number compared to OV, but this is not significant in CL. Bone volume in conjugate-treated groups is comparable to or exceeds that of PG. *p<0.05 as indicated, **p<0.05 compared to each of the other groups. Mean ± SD.

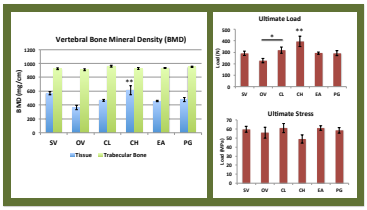


Fig 6. *Left:* Volumetric BMD computed from microCT for the 6th lumbar vertebrae. BMD was computed for the secondary spongiosa including marrow space (Tissue), and trabeculae only (Trabecular Bone). Increase in BMD was not significant for CL. *Right:* Vertebral compression results. *p<0.05 as indicated, **p<0.05 compared to each of the other groups. Mean ± SD.

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 & Fig 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*).

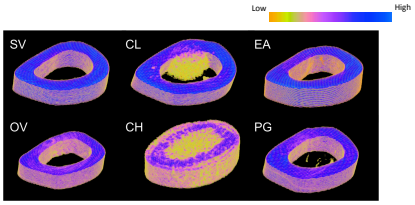


Fig 7. MicroCT images of 1mm thick section in femoral mid-diaphysis scanned at 11.6μm voxel size, with color rendering for density.

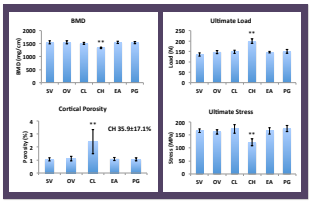


Fig 8. *Left:* MicroCT results for femoral mid-diaphysis. *Right:* Mechanical properties for three-point-bending test. **p<0.05 compared to each of the other groups. Mean ± SD.

DISCUSSION

- Conjugate treatment promoted trabecular bone formation in a dose dependent manner, and increased both osteoblast recruitment (MS/BS) and activity (MAR). This resulted in elevated trabecular bone volume by addition of new trabeculae, which improved the tissue-level BMD in the high dose group but did not significantly affect bone strength, possibly due to short treatment duration.
- 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.

References: [1]Black DM et al. *NEJM.* 2005;353:555-565. [2]Neer RM et al. NEJM. 2001;344:1434-1441. [3]Vahle JL et al. Toxicological Pathology. 2002;30:312-321. [4]Ke HZ et al. Bone. 1998;23:249-255. [5] Mori S et al. Calcified Tissue International. 1992;50:80-87. [6]Mori S et al. Bone. 1990;11:103-113. [7]Ke HZ et al. Bone. 1991;12:173-183. [8]Lee YF et al. Bone. 1995;17:549-554. [9]Jee WSS. Bone. 1990;11:253-266. [10]Yoshida K et al. PNAS. 2002;99:4580-4585. [11]Machwate et al. *Molecular pharmacology.* 2001;60(1):36-41. [12]Graham S et al. Expert Opinion on Investigational Drugs. 2009;18:749-766. [13]Tanaka M et al. Bone. 2004;34:940-948. [14]Ke HZ et al. Journal of Bone and Mineral Research. 2006;21:565-575. [15]Arns S et al. Bioorganic & Medicinal Chemistry. 2012;20:2131-2140.

Acknowledgements: We would like to thank Adeline Ng, Tom Willett, Jenn Baksh, David Josey, Tiffany Ho, and Richard Cheung for their kind assistance in this study. This study was funded by a grant from the Canadian Institute of Health Research (CIHRP). CL was supported by a scholarship from the Ontario Student Opportunities Trust Fund (OSOTF). There are no conflicts of interests to disclose.