The Osteoinductive Effects of Cyplexinol in the Effective Management of Osteoporosis: A Case Study

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Abstract

Osteoporosis, one of the most common metabolic bone diseases, is a systemic skeletal disease characterized by a loss of bone mass and an increased risk of bone fracture, caused by an imbalance in bone resorption and bone mineral deposition (ie, osteogenesis). Although bisphosphonates and other drugs have been shown to possess beneficial therapeutic effects in managing osteoporotic lesions, these drugs can cause significant deleterious side effects. Cyplexinol is a novel, natural protein complex containing osteogenic cytokines and growth factors. Previous studies have shown that it possesses clinically significant pro-osteogenic effects in the joints of patients with osteoarthritis. Specifically, Cyplexinol contains cytokines that inhibit osteoclastogenesis and growth factors that support cellular maturation and proliferation in the bone. To extend these studies and evaluate the effects of Cyplexinol in treating osteoporosis, Cyplexinol was orally administered to a postmenopausal woman suffering from osteoporosis. Although additional studies are required to evaluate the effectiveness of Cyplexinol in a larger cohort, in this case study the author found a statistically significant increase of 51.5% in bone mineral density after 34 mo of Cyplexinol administration, as determined by dual energy x-ray absorptiometry (DXA). Taken together with the osteogenic effects of the Cyplexinol complex as documented in preclinical studies, these results suggest that Cyplexinol may have a role in managing loss of bone mineral density during osteoporosis.

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Osteoporosis is a systemic skeletal disease marked by progressive loss of bone mineral (calcium) and tissue and is a major health threat, afflicting over 44 million people in the United States.[1] Some studies have shown that 68% of those afflicted by osteoporosis are women, indicating that they may be more predisposed to developing the disease than men.[1] Other risk factors associated with increased osteoporosis risk include aging, Caucasian or Asian ethnicity, and a family history of the disease. The early stages of loss of bone mineral density, termed osteopenia, and the initial stages of osteoporosis are not associated with a marked course of symptoms. Loss of bone mineral density occurs and often progresses until a bone fracture occurs.

Osteoporosis is diagnosed when bone mineral density falls significantly below the mean peak bone mass of young healthy adults and is evaluated using dual energy x-ray absorptiometry (DXA).[2] Treatment for osteoporosis involves long-term supplementation with calcium and vitamin D, bisphosphonate administration, and lifestyle changes such as increased exercise and smoking cessation.[3,4] Although bisphosphonate administration represents the most promising mode of osteoporosis treatment, rare but serious adverse events such as osteonecrosis of the jaw, gastrointestinal intolerance, and femur fractures have been reported.[5-7] As a result, other treatment strategies need to be developed that inhibit or reverse the pathogenesis of osteoporosis. Recent research has shown that combinations of teriparatide—a recombinant N-terminal fragment of parathyroid hormone (PTH) that promotes osteogenesis—and denosumab—a monoclonal antibody that inhibits bone loss—can be significantly effective in ameliorating osteoporosis; however, bisphosphonates continue to be the standard of care.[8]

Normal bone growth and remodeling require effective regulation of bone resorption, in which osteoclasts break down bone tissue, as well as of new bone formation, in which osteoblasts mediate osteogenesis.[9] Key to the

Table 1. DEXA Scan of the Lumbar Spine Vertebrae (L1-L4) of a Postmenopausal Woman

Time of DEXA Scan (mo)	Time Posttreatment (mo)	L1	L2	L3	L4	Average
0	-	-2.3	-1.3	-1.8	-2.4	-2
26	-	-2.5	-2	-2.5	-2.6	-2.4
53	0	-3.4	-3.5	-3.2	-3	-3.3
61	8	-3.3	-3.1	-2.9	-2.6	-3
87	34	-2.1	-1.8	-1.5	-0.9	-1.6

proper regulation of bone remodeling is estrogen, in which osteoblasts and peripheral T cells suppress the production of osteoclastogenic cytokines, such as tumor necrosis factor-α (TNF-α).[10,11] Recent research has also underscored a direct role for estrogen in preventing bone loss by promoting Fas ligand upregulation in osteoblasts to induce apoptosis in osteoclasts.[12,13] Menopause-associated deficiencies in estrogen have been shown to increase the rates of bone resorption, which may partially explain why postmenopausal women are prone to developing osteoporosis.[14,15]

Other studies have also shown that deregulation of PTH production, often a result of age-related hyperparathyroidism, may lead to aberrant calcium metabolism and osteoporosis due to increased calcium release from bone tissue.[16,17] PTH increases the activity of 1-α-hydroxylase, the enzyme that directly synthesizes the active form of vitamin D, and vitamin D promotes dietary calcium absorption in the intestine. Therefore, it is tempting to speculate that vitamin D deficiency may lead to secondary hyperparathyroidism and deregulation of PTH production, which in turn leads to osteoporosis.[16] Although several studies have shown an association between vitamin-D deficiency and osteoporosis, others have shown that aberrant PTH production and osteoporosis can occur independently of vitamin-D deficiency.[16-20]

In addition to being caused by pathological bone resorption, osteoporosis can also be caused by diminished osteogenesis.[9] Indeed, aberrations in the osteogenic differentiation of the precursors of mesenchymal stem cells (MSCs) into osteoblasts can lead to osteopenia and osteoporosis.[9,21] Differentiation of MSC precursors into osteoblasts is regulated by certain members of the family of transforming growth factor-β (TGF-β) proteins, called bone morphogenetic proteins (BMPs). Growth factors that support the maturation of newly generated osteoblasts include (1) basic fibroblast growth factor (bFGF), (2) insulin-like growth factor-1 (IGF-1), (3) vascular endothelial growth factor (VEGF), (4) steroids, and (5) TNF-α.[22-29] These osteoinductive signals allow for bone-tissue or matrix synthesis and osteoblast differentiation and subsequent bone deposition and mineralization.[9]

In examinations of the role of IGFs and BMPs in osteogenesis, inhibition of IGF-2 or BMP-2/4/6/9 signaling has been shown to inhibit osteogenic differentiation of osteoblasts, resulting in inefficient bone deposition.[30-37] Moreover, systemic treatment of osteoporotic mice with bFGF, TGF-β, or BMP-2/6 significantly increased bone mineral density and bone tissue deposition and mineralization in several preclinical models of osteoporosis and osteopenia.[32,38,39] Other studies have also shown significant bone healing and osteoinductive effects for BMP-2/7 treatment in bovine models of osteoporosis and osteopenia.[40,41] Taken together with preclinical studies showing that oral administration of bone protein complexes promoted bone formation and biomarkers of bone growth,[36] these data support a potential role for Cyplexinol in the treatment of bone density loss during osteoporosis.

It has previously been shown that Cyplexinol—an osteoinductive protein complex containing bone- and cartilage-stimulating proteins like BMPs, TGF-β, bFGF, and IGF—significantly reduced the incidence and pain associated with osteoarthritis.[42] To evaluate the osteoinductive effects of Cyplexinol, the author set out to determine if its oral administration could improve bone mineral density compared to the current, established standard of care.

The Case Study

To evaluate the osteoinductive effects of Cyplexinol in treating osteoporosis, a 59-year-old female, nonsmoker who was diagnosed with menopause-associated osteoporosis at a community health center was recruited. The woman had been using calcium and vitamin D supplements but continued to lose bone mass progressively for unknown reasons. She was not receiving standard treatment for osteoporosis, such as bisphosphonates; was not using natural or synthetic steroids; and was not undergoing hormone replacement therapy. The woman also had no history of type 1 or type 2 diabetes, Paget's disease, primary hyperparathyroidism, or rheumatoid arthritis.

A bone mineral density scan was conducted using DXA (Hologic, Boca Raton, FL, USA), with standard methodological approaches. As evidenced by three initial DXA scans of her spine's lower lumbar region at months 0, 26, and 53, the woman exhibited a progressive loss of bone mineral density consistent with a progression from osteopenia to osteoporosis (Table 1). After the last of the three scans

at month 53, she began taking two Ostinol tablets orally each day, one in the morning and one in the evening, and she continued this regimen from March 2009 to January 2012, almost 3 years. Each tablet contained 200 mg of Cyplexinol, a natural, partially hydrolyzed, collagen complex containing BMPs, TGF-β, bFGF, VEGF, and IGF. DXA scans were performed to evaluate her bone mineral density after initiation of Cyplexinol treatment at 8 and 34 months.

The *t* scores were computed as the number of standard deviations that the values from the experimental DXA scan were above or below the normative mean values of healthy 30-year-old women. The *t* scores were averaged, and statistically significant differences over time were determined by evaluating the significance of the differences in *z* scores, based on a normal distribution and hypothesis testing using an α value of 0.05 (Graphpad Prism, Lebanon, NJ, USA).

Results

Based on the *t* scores from the DXA scans, a statistically significant increase occurred 34 months after the subject began taking Cyplexinol (Table 1). As determined by analyzing the *t* score of the DXA scan performed 8 months after the start of Cyplexinol treatment, a 10% increase in bone density was observed, which correlated with the cessation of a 53-month history of bone loss. As evidenced by the *t* scores of DXA scans at the start of Cyplexinol treatment (mo 53) and after 34 months of treatment, a 51.5% increase in bone mineral density occurred, resulting in an osteoporotic spine becoming osteopenic. Further, at onset of treatment with Cyplexinol the L4 vertebrae *t* score was at -3.0, indicating moderate to severe osteoporosis. After 34 months of treatment, the L4 vertebrae t-score improved to -0.9, indicating normal bone density.

Discussion

Given the osteogenic effects of BMPs, it has been postulated that oral administration of Cyplexinol would reduce the loss of bone mineral density and increase bone mineral deposition. Indeed, the research performed by Garian et al had previously shown that Cyplexinol administration had beneficial, osteoinductive effects in the effective treatment of moderate and severe osteoarthritis in humans.[42]

Taken together with the roles for BMPs and TGF-β in promoting osteoblast differentiation and the proliferative effects of growth factors like bFGF, VEGF, and IGF, the woman's increased bone mineral density following Cyplexinol administration in the current case study was likely due to increased osteoblast-mediated, bone mineral deposition. This assessment is consistent with preclinical studies evaluating the therapeutic roles for BMP-2/6, bFGF, and TGF-β in treating osteoporotic lesions.[32,38,39] Since Cyplexinol contains BMPs and growth factors like bFGF and IGF-2, it is capable of targeting multiple pro-osteogenic pathways in a manner similar to a polypharmacological approach. The cumulative effects of the constituent proteins in the Cyplexinol complex were likely additive in nature but may have synergistically boosted osteogenic differentiation of MSCs in the spine. Although this case study indicates potential for Cyplexinol in reversing loss of bone mineral density during the development of osteoporosis, larger, randomized clinical trials of Cyplexinol need to be conducted to determine whether the supplement has a significant beneficial effect in treatment of osteoporosis. Additionally, data need to be compared to a control population to evaluate the full extent of Cyplexinol's osteogenic effects.

Conclusion

In summary, these data suggest that Cyplexinol may have a role in managing loss of bone mineral density during osteoporosis. Although it appears that long-term studies may be needed to observe effects, this case study is the first clinical case study evaluating the efficacy of Cyplexinol's protein complex in managing osteoporosis. Taken together with preclinical studies documenting the osteogenic effects of the component growth factors and cytokines in Cyplexinol's protein complex, these results highlight a potentially new, orally bioavailable therapeutic for the effective treatment of osteoporosis.

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