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Selective osteogenesis by a synthetic mineral inducing peptide for the treatment of osteoporosis

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ABSTRACT

Mineralization in mammalian cells is accomplished by concerted regulation of protein-based extracellular matrix (ECM) components, such as non-collagenous proteins and collagen fibrils. In this study, we investigated the ability of a collagen-binding motif (CBM) peptide derived from osteopontin to selectively affect osteogenic or adipogenic differentiation *in vitro* and *in vivo*. In particular, increased osteogenic differentiation and decreased adipogenic differentiation were observed in human mesenchymal stem cells (hMSCs). Osteocalcin (OCN) protein expression in MC3T3-E1 cells without osteogenic inducers was then investigated following treatment with the CBM peptide. In ovariectomized (OVX) mice, estrogen deficiency induced osteoporosis and increased fat tissue deposition. However, after the CBM peptide or estradiol was injected into the OVX mice for 2 months, the increased serum OCN concentration and alkaline phosphate (ALP) activity were decreased in the estradiol-treated group (OVX-E) and the high-concentration CBM peptide-treated group (OVX-HP). Significant bone loss was also observed in the ovariectomized mice (OVX-PBS). In particular, the bone volume per total volume (BV/TV) and bone mineral density (BMD) were significantly decreased in the OVX mice; however, both of these markers were restored in the OVX-HP group, which also had significantly well-developed bone structure and bone formation. In contrast to the bone structural change, adipose tissue was increased in the OVX-PBS. However, a significant decrease in total fat and subcutaneous fat was observed in the low-concentration CBM peptide-treated group (OVX-LP) and the estradiol-treated group (OVX-E). Taken together, these results suggest that the CBM peptide could be an effective therapeutic agent for osteoporosis due to its selective stimulation of osteogenic differentiation, rather than adipogenesis.

1. Introduction

Osteoporosis is a common degenerative disease that causes bones to become abnormally thin, easily fragile, and more likely to fracture due to an imbalance in bone absorption and replacement [1]. To prevent and cure bone loss, there are medications available to reduce the risk of broken bones. These medicines either slow or stop bone loss or rebuild bones. Most current osteoporosis treatments are anti-resorptive, inhibiting osteoclastic bone resorption but not promoting new bone formation [1]. Anti-resorptive medicines, including bisphosphonate, alendronic acid, alendronate (Fosamax), ibandronate (Boniva), risedronate (Actonel), and zoledronic acid (Aclasta), have been widely used in the clinic to reduce the risk of broken bones in the spine and, in certain cases, the hip. However, these treatments are likely to cause several side effects, including nausea, abdominal pain, and a risk of an inflamed esophagus or esophageal ulcers [2–4]. Therefore, there is a need for a bone-regenerative therapy that can reduce the risk of osteoporotic fractures by increasing bone formation, with greater safety, less toxicity, and greater therapeutic effects than anti-resorptives.

Osteoporosis due to postmenopausal estrogen deficiency most likely results from the replacement of cancelous bone by bone

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marrow adipose tissue. Recent studies suggest that alterations in bone formation and adipogenesis during aging may be a consequence of enhanced differentiation of osteoprogenitor cells into adipocytes and reduced differentiation into osteoblasts [5–7]. This association is further supported by the fact that bone marrow adipocytes share a common progenitor cell with bone-forming osteoblasts [8]. Furthermore, estrogen has been implicated in a reciprocal relationship between bone marrow adipocytes and osteoblasts. In addition, there is evidence that estrogen preferentially promotes the formation of osteoblasts instead of adipocytes [9]. Therefore, hormone replacement therapy (HRT) has been considered as a major prophylactic and treatment for osteoporosis resulting from estrogen deficiency. However, evidence suggests that HRT might also be associated with an increasing prevalence of cancer in postmenopausal women [10]. Therefore, alternative therapeutic drugs that can correct the imbalance between osteogenesis and adipogenesis in osteoporotic disorders and increase bone mineral density (BMD), as the activity of estrogen does, should be explored.

In a previous study, a collagen-binding motif (CBM) peptide (GLRSKSKKFRRPDIQYPDA) was newly identified in osteopontin and chemically synthesized [11]. This CBM peptide can bind to collagen directly, without a chemical conjugation reaction, because of its binding specificity for the collagen surface. Although the collagen surface alone is not able to induce remarkable mineral deposition, the collagen-CBM complex significantly induces mineral creation *in vitro*. More specifically, osteogenic differentiation of human mesenchymal stem cells (hMSCs) was achieved in mineralization media with the CBM peptide over a 14-day culture period. The CBM peptide was also able to induce bone formation, especially in the early phase of osteogenesis, when applied to the biomaterial *in vivo* [11]. Development of the CBM peptide as an anti-osteoporotic agent is anticipated if the CBM peptide is additionally able to specifically induce osteogenesis by recovering the osteogenic/adipogenic balance *in vitro* and *in vivo*.

In the present study, we investigated whether the activity of the CBM peptide was able to stimulate the osteogenic or adipogenic differentiation of hMSCs and to differentiate preosteoblasts into osteoblasts in complete media. The therapeutic anti-osteoporotic activity of and the inhibition of fat tissue deposition by the CBM peptide in an ovariectomized mice model were also examined.

2. Materials and methods

2.1. Materials

Dulbecco's modified essential medium (DMEM), α-minimal essential medium (α-MEM), Hank's balanced salt solution (HBSS), trypsin-EDTA, fetal bovine serum (FBS), and antibiotic-antimycotic solution were purchased from GibcoBRL (Grand Island, NY, USA). Ascorbic acid, β-glycerophosphate, dexamethasone, 17β-estradiol, and bovine serum albumin (BSA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). The other chemicals used were of analytical grade. Human bone marrow-derived MSCs were ordered from LONZA (Walkersville, MD, USA).

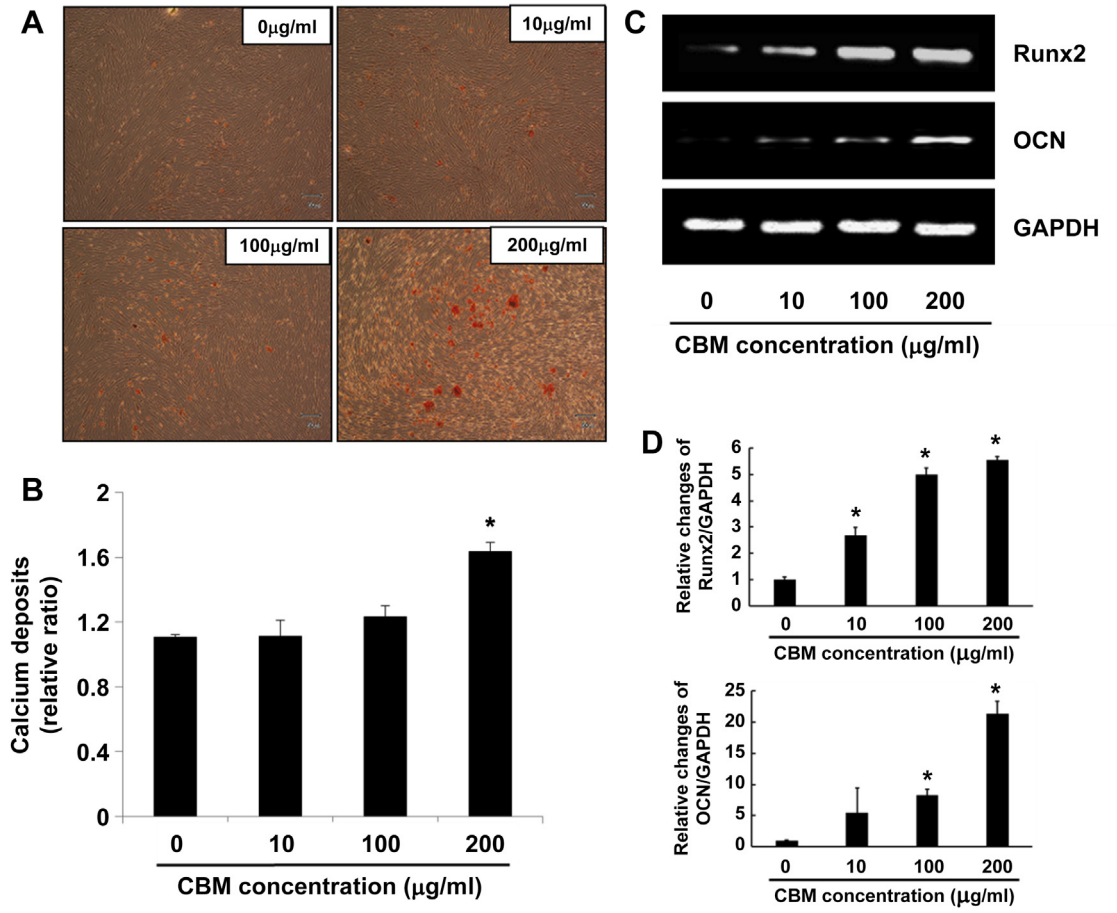

Fig. 1. Osteoblastic differentiation in hMSCs induced by the CBM peptide. Cells were grown in osteogenic media in the presence or absence of the CBM peptide (0, 10, 100, or 200 μg/ml). After 14 days, the cells were fixed and stained with alizarin red S. (A) Differentiated stem cells positive for alizarin red S were stained red. (B) The calcium deposits were quantified. (C) The effect of the CBM peptide on osteogenic-specific gene expression was detected by RT-PCR. (D) The expression levels were quantified. GAPDH was amplified as a loading control. The data are expressed as the mean ± SD from three independent experiments in each group. *$p < 0.05$ vs. the untreated control groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)