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EXPERIMENTAL STUDY

Algal oligosaccharides ameliorate osteoporosis *via* up-regulation of parathyroid hormone 1-84 and vascular endothelial growth factor

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Abstract

OBJECTIVE: To determine whether algal oligosaccharide affects the levels of parathyroid hormone 1-84 (PTH1-84) and vascular endothelial growth factor (VEGF).

METHODS: An osteoporosis rat model was established *via* bilateral ovariectomy. The model rats were fed algal oligosaccharides (molecular weights: 600-1, 200 Da) for 4 months. Bone mineral density (BMD) was then measured. MG-63 human osteoblastic cells were treated with algal oligosaccharides. The expression of PTH1-84 and VEGF was then examined. Oligosaccharide-treated cells were transfected with PTH1-84 short hairpin RNA (shRNA), VEGF shRNA, and PTH1-84-VEGF small interfering RNA (siRNA). The growth rates were then compared between transfected and non-transfected cells.

RESULTS: Algal oligosaccharides increased the BMD of the osteoporosis rat model compared with untreated controls ($P < 0.05$). When MG-63 cells were treated with algal oligosaccharides, the growth rate increased by 25% compared with the control group at day 3 ($P < 0.05$). In addition, the expression of PTH84 and VEGF was enhanced. Conversely, when the cells were transfected with PTH84 shRNA, VEGF shRNA, or PTH1-84-VEGF siRNA, the growth rate was decreased by 17%, 35% and 70%, respectively, compared with controls at day 3 ($P < 0.05$).

CONCLUSION: Algal oligosaccharides ameliorate osteoporosis *via* up-regulation of PTH1-84 and VEGF. Algal oligosaccharides should be developed as a potential drug for osteoporosis treatment.

Key words: Osteoporosis; Bone density; Sargassum; Oligosaccharides; Parathyroid hormone; Vascular endothelial growth factor A; MG-63 human osteoblastic cells

INTRODUCTION

Over 200 million people suffer from osteoporosis worldwide. The prevalence of osteoporosis is still increasing, especially in the elderly population.[1] Osteoporosis can increase the possibility of fragility fractures[2] that lead to morbidity, mortality, and decrease quality of life. Alendronate sodium,[3] risedronate sodium,[4] zoledronic acid,[5] bisphosphonates,[6] calcitonin,[7] denosumab,[8] and estrogen[9] are drugs for the treatment of osteoporosis. However, all of these drugs have unwanted adverse effects or high costs.[10-12] Therefore, it is critical to explore low-cost medicine with few side-effects for osteoporosis treatment.

Algal oligosaccharides are an abundant resource and have attracted increasing attention for their active components and various therapeutic functions.[13] More importantly, algal oligosaccharides are safe without toxicity or side-effects.[14] Algal extracts have been reported to increase bone density and ameliorate osteoporosis. The

main components of the extracts are saccharides[15,16] that can be digested into pharmaceutical oligosaccharides. Therefore, algal oligosaccharides may be a potential drug for the treatment of osteoporosis, but the molecular mechanism remains unknown.

Parathyroid hormone (PTH), an important modulator of bone homeostasis,[17] is linked to the development of stress fractures in bone. Human PTH1-84 is a member of the parathyroid hormone family, which enhances calcium reabsorption and promotes bone formation.[18] Comparatively, vascular endothelial growth factor (VEGF), a growth factor for endothelial cells, has been shown to play major roles in bone health and is associated with bone mineral density (BMD).[19] On the other hand, PTH1-84 has been reported to increase endothelium-dependent vasodilation of the artery via enhanced nitric oxide production and is partially dependent on VEGF signaling.[20] Therefore, we determined whether algal oligosaccharides can ameliorate osteoporosis *via* regulation of PTH1-84 and VEGF.

MATERIALS AND METHODS

Reagents and cell culture

Algal oligosaccharides mainly consisting of trimer to hexamer oligosaccharides (molecular weights: 600-1, 200 Da) were chosen according to a previous report.[21] Algal oligosaccharides were purchased from Qingdao BZ Oligo Biotech Co., Ltd. (Qingdao, China). MG-63 human osteoblastic cells (Cell Bank, Shanghai Institutes for Biological Sciences, Shanghai, China) were grown in Roswell Park Memorial Institute 1640 medium with 10% heat-inactivated fetal bovine serum (Shanghai ExCell Biology, Shanghai, China). Cells were cultured at 37 ℃ in a CO_2 incubator with saturated humidity. The cells were randomly assigned to the experimental group (treated with 5 μg/mL algal oligosaccharides for 3 days) or control group (untreated).

Toxicity testing

The toxicity of algal oligosaccharides was tested by the viability of MG-63 cells. The viability of MG-63 cells was measured by trypan blue uptake and [^{3}H]thymidine incorporation after the cells were treated with various concentrations of algal oligosaccharides (0, 5, 10, 15, 20, 25, 30, 35, and 40 μg/mL) for 24 h. The number of viable cells was counted by trypan blue exclusion in two microscopic fields. Results are shown as the percentage of viable cells. For [^{3}H]thymidine incorporation assays, after exposure to various concentrations of algal oligosaccharides, 200 μL of a cell suspension (1×10^5 cells/mL) was transferred to each well of a 96-well plate (Beijing South Star Paper Product Co., Ltd., Beijing, China). After 24 h, the cells were treated with 1 Ci/well [^{3}H]thymidine (67 Ci/mmol) for 4 h and then collected with an automated sample harvester (Skatron, Sterling, VA, USA), dried, and analyzed in an Aquassure (Packard, Meriden, CT, USA).

Animals and treatments

Healthy 3-month-old female Sprague-Dawley rats [(220 ± 20) g] were obtained from Shanghai Sipprbk Lab. Animals Ltd. (Shanghai, China). All rats were adapted to the environment for 1 week before the start of experiments. The acclimated rats underwent either bilateral laparotomy (sham, n = 12) or bilateral ovariectomy (OVX, n = 24) according to a previous report.[22] One month after recovering from surgery, OVX rats were randomly assigned into two groups: OVX treated with the vehicle (OVX, n = 12) and OVX treated with algal oligosaccharides (oral, 5 $mg \cdot kg \cdot^{-1} \cdot d^{-1}$, n = 12). Treatments were initiated at 1 month after OVX and proceeded for 4 months. The body weights of the rats (in grams) were measured for every 7 days. Before sacrifice, each rat was housed in a metabolic cage without food for 1 day. After laparotomy of anesthetized rats with pentobarbital sodium (30 mg/kg), blood samples were collected by abdominal aorta puncture. Then, serum specimens were collected by centrifugation at 1500 ×g for 20 min. Serum samples were stored at − 20 ℃ until analysis. Femurs and the fourth lumbar (L4) vertebrae were dissected for measurement of BMD.

BMD measurement

The BMD of L4 vertebrae and right femurs was estimated using dual-energy X-ray absorptiometry (DEXA, GE Healthcare, Seattle, WA, USA). The measurements are expressed as grams of mineral content per cm^2 of bone surface. Scans were performed by the same operator.

Enzyme-linked immunosorbent assay (ELISA)

PTH1-84 and VEGF in serum samples were measured by ELISA. ELISA kits for PTH1-84 (Cat. No. S-1226) and VEGF (Cat. No. KHG0111) were purchased from Bachem Holding AG (Basel, Switzerland) and Life Technologies (Beijing, China).

PTH1-84 and VEGF gene silencing

The pTZU6+1 plasmid was a gift from Shanghai Boya Biological Engineering Technology (Shanghai, China). Small interfering RNA (siRNA) sequences used for PTH1-84 and VEGF gene silencing were as follows: siPTH1-84, sense 5′-TCGA GTAAACCTGGGG-TAGGTGGGGGTTCTGTCTTAACTCGAGGGG-CTTTTTT-3′ , antisense 5′-CTAGAAAAAAGCCC-CTCGAGTTAAGACAGAACCCCCACCTACCCC-A GGTTTAC-3′; siVEGF, sense 5′-TCGAGCGGAT-CAAACCTCACCAAGGCCAGCAC ATAGGAGA-GATGAGCTTTTTT-3′ , antisense 5′-CTAGAAAA-AAGCTCATCTCTCCTATGTGCTGGCCTTGTG-AGGTTTGATCCGC-3′. pTZU6+1-shRNA- PTH1-84 and pTZU6+1-shRNA-VEGF plasmids were constructed. To explore the effect of RNA interference of both PTH1-84 and VEGF on MG-63 cells, the pGenesil-2.1 plasmid was purchased from Wuhan Genesil Biotechnology Co., Ltd. (Wuhan, China). Fu-