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Lycopene treatment against loss of bone mass, microarchitecture and strength in relation to regulatory mechanisms in a postmenopausal osteoporosis model☆

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ABSTRACT

Lycopene supplementation decreases oxidative stress and exhibits beneficial effects on bone health, but the mechanisms through which it alters bone metabolism *in vivo* remain unclear. The present study aims to evaluate the effects of lycopene treatment on postmenopausal osteoporosis. Six-month-old female Wistar rats (n = 264) were sham-operated (SHAM) or ovariectomized (OVX). The SHAM group received oral vehicle only and the OVX rats were randomized into five groups receiving oral daily lycopene treatment (mg/kg body weight per day): 0 OVX (control), 15 OVX, 30 OVX, and 45 OVX, and one group receiving alendronate (ALN) (2 μg/kg body weight per day), for 12 weeks. Bone densitometry measurements, bone turnover markers, biomechanical testing, and histomorphometric analysis were conducted. Micro computed tomography was also used to evaluate changes in microarchitecture. Lycopene treatment suppressed the OVX-induced increase in bone turnover, as indicated by changes in biomarkers of bone metabolism: serum osteocalcin (s-OC), serum N-terminal propeptide of type 1 collagen (s-PINP), serum crosslinked carboxyterminal telopeptides (s-CTX-1), and urinary deoxypyridinoline (u-DPD). Significant improvement in OVX-induced loss of bone mass, bone strength, and microarchitectural deterioration was observed in lycopene-treated OVX animals. These effects were observed mainly at sites rich in trabecular bone, with less effect in cortical bone. Lycopene treatment down-regulated osteoclast differentiation concurrent with up-regulating osteoblast together with glutathione peroxidase (GPx) catalase (CAT) and superoxide dismutase (SOD) activities. These findings demonstrate that lycopene treatment in OVX rats primarily suppressed bone turnover to restore bone strength and microarchitecture.

1. Introduction

Postmenopausal osteoporosis is a serious public health concern associated with significant morbidity, mortality, deterioration of quality of life, and high health care costs [1]. Current FDA-approved therapies include antiresorptive (e.g., bisphosphonate and denosumab) and anabolic (e.g., teriparatide) agents [2]. While denosumab is relatively new and unstudied, bisphosphonate and teriparatide therapies have contributed to a documented decrease in fracture risk among treated patients [3]. However, the potentially undesirable side effects associated with these pharmacological therapies, including atypical fractures, osteonecrosis of the jaw, gastro-oesophageal adverse events, and dizziness, along with poor compliance and cost concerns for teriparatide, continue to challenge their overall efficacy [4,5]. Thus, research efforts are directed towards discovering more effective, lower-cost therapeutic strategies, including natural alternatives with minimal side effects and fewer compliance challenges.

There is growing evidence that oxidative stress, induced by reactive oxygen species (ROS) that increase with aging or with the onset of an inflammatory state, can adversely affect bone homeostasis [6]. Recent studies have suggested that postmenopausal bone loss may be caused by ROS, which induce a more oxidized bone microenvironment [7], and an excess of ROS may inhibit osteoblast differentiation and proliferation [8]. ROS generated in the extra- or intra-osteoclasts act as signals to enhance osteoclastic differentiation, resulting in more bone resorption [9,10]. Thus, an imbalance in ROS levels can accelerate bone

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resorption, resulting in bone fragility and fracture: accordingly, eliminating excessive ROS is an effective approach for maintaining bone integrity [11].

Estrogen deficiency, an independent risk factor for bone fragility [12], has been linked to an increase in oxidative stress. Ovariectomy in rats induces oxidative stress and attenuates levels of antioxidants [13]. In addition, bone marrow of OVX rats exhibits increased ROS levels and decreased activity of antioxidative enzymes (e.g., glutathione reductase) [14]. In a mouse model, higher levels of the antioxidant glutathione prevent bone loss during estrogen deficiency, whereas depletion of antioxidants increases bone loss [15]. Moreover, in OVX rats, plasma lipid peroxidation levels increase as compared with sham-operated controls [16,17]. In postmenopausal women, Bednarek-Tupikowksa et al. demonstrated higher serum lipid peroxide levels and lower antioxidative potency (indicated by decreased glutathione levels and glutathione peroxidase (GPx) activity), as compared with premenopausal women [18]. Taken together, these studies suggest that estrogen deficiency-induced bone loss is accompanied by higher local and systemic oxidative stress.

Dietary supplementation or treatment with antioxidants is an effective approach to counteract and ameliorate excessive ROS production. Lycopene, a carotenoid found in red fruits and vegetables, especially tomatoes and tomato products, is one of the most potent antioxidants, with a scavenging capacity for singlet oxygen molecules 100 times higher than that of other carotenoids [19], thus, it has been associated with a decreased risk of chronic diseases (for review see [20]). Ben-Dor et al. [21], proposed that the effect of lycopene may be attributed to the induction of anti-oxidant and phase II enzymes. The transcriptional up-regulation of the genes encoding such enzymes is mediated by cis-acting DNA sequences located within their promoter regions known as anti-oxidant response elements (AREs). The major ARE transcription factor is Nrf2 (nuclear factor E2-related factor 2). It plays key roles in the detoxification processes and modulation of anti-oxidant cellular defense system promoting the up-regulation of stress-induced cyto-protective enzymes [e.g., superoxide dismutase (SOD), GPx, heme oxygenase 1 (HO-1)] [22]. The Nrf2 is normally sequestered in the cytoplasm by Kelch-like ECH-associated protein-1 (Keap1). Oxidative stress promotes the dissociations of Nrf2 from Keap1, translocates to the nucleus and complexes with other factors and binds to AREs to regulate the expression of target genes [23,24]. In addition, Nrf2 also elicits anti-inflammatory effects. Lycopene and its metabolites were found to mediate the activation of Nrf2-ARE signaling and subsequent induction of gene expression [25]. Moreover, lycopene might target other signaling pathways for its cellular anti-oxidant actions (for review see [26]).

Recently, Mackinnon et al. showed that a lycopene-restricted diet significantly decreased circulating lycopene and decreased the antioxidant enzymes SOD and catalase (CAT) in healthy postmenopausal women [27]. In addition, the same group showed in a pilot study using a small group of healthy postmenopausal women that lycopene supplementation significantly decreased oxidative stress markers and a bone resorption marker [28]. In OVX rats, lycopene supplementation prevents bone loss and restores bone strength [29,30]. However, how lycopene exerts its bone-protective effects remains uncertain.

To provide more insight into the possible mechanisms of the bone-sparing effects of lycopene, we investigated the effect of lycopene treatment on bone loss in an OVX rat model. We comprehensively assessed bone health with measurements of bone turnover markers (BTMs), bone mass, bone dynamics, bone microarchitecture, and bone strength parameters. In conjunction, we evaluated alterations in regulators of oxidative stress and of osteoblast and osteoclast differentiation and activity at the tissue level. Additionally, we used alendronate (ALN) treatment as a positive control to compare the bone metabolic response to lycopene treatment with that of an established antiresorptive therapy.

2. Subjects and methods

2.1. Animals and experimental design

A total of 264 Wistar female rats, ~6 months old, were supplied by the Animal House at King Fahd Medical Research Center (KFMRC), KAU. Rats were housed in individual cages and maintained at 22 °C with a 12-h light/dark cycle. During the study period, rats were given standard rodent chow diet *ad libitum* (commercial rat cubes containing approximately 18% protein, 3% fat, 77% carbohydrate, and 2% of an inorganic-salt mixture with a vitamin supplement by weight, supplied by Grain Silos and Flour Mills Organization, Jeddah, Saudi Arabia) and water until the onset of intervention. Prior to surgery, rats were anesthetized with ketamine [10% ketamine hydrochloride (80 mg/kg body weight), Cayman Chemical, Michigan, USA] and xylazine [(2% xylazine hydrochloride, 10 mg/kg body weight), Sigma-Aldrich Co., St. Louis, USA]. A single longitudinal skin incision was made on the dorsal midline at the level of the kidneys and both ovaries were ligated and removed to produce an ovariectomy in all OVX group animals. A SHAM-operated group, acting as a negative control for the effect of ovariectomy on bone parameters, underwent bilateral laparotomy, during which the ovaries were exposed but remained intact [31]. In accordance with a previously published study [32], vaginal smears were collected 3–10 days after surgery to confirm the effects of the surgery: all groups (except the SHAM group) demonstrated successful ovariectomy. The lycopene-supplemented groups were given lycopene (15, 30 and 45 mg/kg body weight per day) dissolved in corn oil by daily intragastric administration for the experimental period of 12 weeks. The SHAM, OVX and ALN control groups were given the same volume of corn oil without lycopene treatment. Our rational for lycopene dose selection is based on previous studies, which have showed lycopene supplementation at these doses, its bioavailability, as well as its protective effect against oxidative stress. In addition, these doses are achievable through diet in human, and the range also conforms to the daily recommended levels of lycopene intake (for review see [33]). We conducted a 12-week randomized placebo-controlled study in order to examine the protective effects of lycopene supplementation on bone mass and fragility in OVX rats. Prior to surgery, body weight and bone mineral density (BMD) were obtained for all animals. Rats were randomized using a computer-generated randomization code and assigned into one of six groups: (1) sham-operated (SHAM; n = 44); (2) ovariectomized control (OVX; n = 44); (3) OVX lycopene-supplemented (15 mg/kg body weight per day) (n = 44); (4) OVX lycopene-supplemented (30 mg/kg body weight per day) (n = 44); (5) OVX lycopene-supplemented (45 mg/kg body weight per day) (n = 44) and (6) OVX alendronate-treated (ALN) [2.0 μg/kg body weight per day subcutaneously (sc)] (n = 44). Alendronate is an antiresorptive agent for the treatment of osteoporosis and was used as a positive therapy control. This dosage was based on preclinical studies that demonstrated significant increases in bone mass and strength [34], and is comparable to the 20 mg/kg/day dosage prescribed to treat osteoporosis. All OVX groups were pair-fed according to the daily food intake of the SHAM group throughout the study to avoid the possible effects of ovariectomy-induced over-consumption and subsequent excessive weight gain [35]. Treatment started 1 week after the surgery: this period of rest allowed the rats to recover from the stress associated with the surgery. In addition, after surgery, rats were administered Diclofenac sodium injection (20 mg/kg body weight) to reduce the pain associated with the surgery. Rats were weighed and examined daily. Blood and urine samples were collected at baseline, during, and at the end of treatment for measurement of various BTMs and other analytes. *In vivo* dual energy x-ray absorptiometry (DXA) measurements were made for the bone mineral content (BMC) and BMD of the whole body, lumbar spine (L_1-L_4), and the right humerus, together with body composition at 12 weeks of treatment. One day following the post-test DXA assessments