Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 20 (2009) 35–44
Journal of Nutritional Biochemistry

Dried plum polyphenols attenuate the detrimental effects of TNF-α on osteoblast function coincident with up-regulation of Runx2, Osterix and IGF-I☆

So Young Bu[a], Tamara S. Hunt[b], Brenda J. Smith[a,b,c,*]

[a]Department of Nutritional Sciences, College of Human Environmental Science, Oklahoma State University, Stillwater, OK 74078
[b]Department of Surgery, College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190
[c]Department of Medicine, College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Received 19 July 2007; received in revised form 25 October 2007; accepted 26 November 2007

Abstract

Previous studies have demonstrated that dried plums which contain high amounts of polyphenols can restore bone mass and structure, and significantly increase indices of bone formation. The purpose of this study was to determine how dried plum polyphenols influence osteoblast activity and mineralized nodule formation under normal and inflammatory conditions. MC3T3-E1 cells were plated and pretreated with dried plum polyphenols (0, 2.5, 5, 10 and 20 μg/ml) and 24 h later stimulated with TNF-α (0 or 1.0 ng/ml). The 5, 10 and 20 μg/ml doses of polyphenols significantly increased intracellular ALP activity under normal conditions at 7 and 14 days, and restored the TNF-α-induced suppression of intracellular ALP activity by 14 days ($P<.001$). Polyphenols also increased mineralized nodule formation under normal and inflammatory conditions. In the absence of TNF-α, 5 μg/ml of polyphenols significantly up-regulated the growth factor, IGF-I, compared to controls, and the 5 and 10 μg/ml doses increased the expression of lysyl oxidase involved in collagen crosslinking. TNF-α decreased the expression of Runx2, Osterix and IGF-I, and polyphenols restored their mRNA levels to that of the controls. Although TNF-α failed to alter lysyl oxidase at 18 h, the polyphenols up-regulated its expression ($P<.05$) in the presence of TNF-α. As expected, TNF-α up-regulated RANKL mRNA and polyphenols suppressed RANKL expression without altering OPG. Based on these findings, we conclude that dried plum polyphenols enhance osteoblast activity and function by up-regulating Runx2, Osterix and IGF-I and increasing lysyl oxidase expression, and at the same time attenuate osteoclastogenesis signaling.

Keywords: Bone; Antioxidants; Osteoporosis

1. Introduction

Normal bone homeostasis is maintained by a balance between bone formation and bone resorption [1]. Conditions in which bone formation by osteoblasts is decreased relative to the activity of bone resorpting osteoclasts result in a net loss of bone mass [2,3]. Osteoblasts, fibroblast-like cells derived from a mesenchymal lineage, synthesize enzymes and matrix proteins involved in the formation of mineralized bone [4] and serve as a source for osteoclast differentiation factors including receptor activator of NF-κB ligand (RANKL) [5]. Inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 decrease osteoblast activity and stimulate osteoblasts to produce inflammatory cytokines such as RANKL, prostaglandin E_2 (PGE_2) and IL-1 that can enhance osteoclast differentiation and activity [6,7].

In gonadal hormone deficiency, TNF-α has been identified as one of the predominant pro-inflammatory mediators of bone loss [8]. TNF-α inhibits osteoblast activity and bone mineralization by down-regulating growth factors such as

☆ This material is based upon work supported by Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, under award No. [2006-35200-17383]. This study was also supported by the California Dried Plum Board and funds from the Oklahoma Center for the Advancement of Science and Technology [HR06-109].

* Corresponding author. Oklahoma State University, Stillwater, OK 74078. Tel.: +1 405 744 3866.
E-mail address: bjsmith@okstate.edu (B.J. Smith).

0955-2863/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.11.012

insulin-like growth factor (IGF)-I [9] and alkaline phosphatase (ALP) [10] involved in the formation of hydroxy apatite, and decreasing the expression of lysyl oxidase, the enzyme responsible for collagen crosslinking [11]. Suppression of osteoblast activity by TNF-α is associated with down-regulation of transcription factors, Osterix and Runx2, which regulate the expression of ALP and IGF-I as well as several bone matrix proteins including osteopontin and bone sialoprotein [12,13]. TNF-α also alters osteoblast signaling by increasing RANKL production which promotes osteoclast differentiation and activity [6]. Under conditions of gonadal hormone deficiency, decreased osteoblast activity and promotion of osteoclast differentiation by inflammatory cytokines such as TNF-α are associated with a defective antioxidant system [14]. Supplementation with antioxidants attenuates ovariectomy-induced bone loss by suppressing TNF-α and enhancing bone formation [15].

Some polyphenolic compounds and their derivatives, which reside in fruits and vegetables, have antioxidant and anti-inflammatory properties that have been shown to influence both osteoclasts and osteoblasts. For example, polyphenols such as caffeic acid, resveratrol and rutin inhibit osteoclast differentiation and activity [16,17], directly stimulate osteoblasts, and favorably alter bone formation markers [18,19]. Caffeic acid, one of the polyphenols in dried plums (*Prunus domestica* L.), has been reported to reverse the oxidative stress (H_2O_2)-induced decrease in ALP and type I collagen expression by osteoblasts as well as the phosphorylation of Runx2 [20]. Resveratrol, the major phenolic compound in grapes, stimulates the proliferation and differentiation of osteoblasts and increases intracellular ALP activity and bone morphogenic protein (BMP-2) expression [19]. Rutin is another polyphenol found in plums [21] and is reported to increase serum osteocalcin and bone mineral density (BMD) in estrogen-deficient osteopenic rats [18]. Findings from these studies suggest that a variety of individual phenolic compounds modulate osteoblast activity and signaling, and that an optimal combination of these compounds may have anabolic effects on bone.

Dried plum, a rich source of polyphenols [21], has been shown to positively influence bone mass, bone microarchitecture and serum markers of bone metabolism [16,22,23]. A short-term study of postmenopausal women consuming approximately 100 g of dried plum per day (i.e., 10–12 dried plums) demonstrated that dried plum increased serum bone-specific ALP and IGF-I [23]. Data from animal studies indicate that dried plum enhances circulating IGF-I in gonadal hormone deficiency models of osteoporosis [22,24] and effectively restored bone in osteopenic ovariectomized female rats [25]. Recently, dried plum's ability to restore bone mass and microarchitecture in osteopenic gonadal hormone-deficient male rats was compared to the anabolic agent, parathyroid hormone (PTH) [26]. Dried plum completely reversed the decrease in bone mass compared to sham-operated control animals and had similar effects to PTH on vertebral trabecular bone architecture and biomechanical properties. Although other plant-based foods with relatively high phenolic compound content such as soy favorably modulate bone metabolism, their ability to restore bone in osteopenic animal models appears to be somewhat limited [27].

Based on the findings from these animal and clinical studies [22–26], we anticipate that components of dried plum such as its polyphenolic compounds mediate these anabolic effects on bone by altering osteoblast signaling, maturation and/or activity. Hence, the purpose of this study was to investigate how polyphenols extracted from dried plum stimulate osteoblast activity and mineralized nodule formation under normal and inflammatory conditions.

2. Methods and materials

2.1. Materials and Reagents

MC3T3-E1 (RIKEN No. RCB1126), mouse calvarial pre-osteoblastic cells were obtained from Riken BioResource Center (Ibaraki, Japan). Fetal bovine serum (FBS) and penicillin G-streptomycin were purchased from GIBCO-BRL (Grand Island, NY, USA). Minimum essential medium (α-MEM), ascorbic acid, β-glycerophosphate, alizarin red-S and mouse TNF-α were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dried plum powder was generously provided by the California Dried Plum Board. For ALP activity measurements, an alkaline phosphatase substrate kit from Bio-Rad (Hercules, CA, USA) was used. Unless otherwise listed, all other chemicals were reagent grade and obtained from Fisher Scientific.

2.2. Experiment 1

The objective of Experiment 1 was to evaluate the effects of polyphenols extracted from dried plum on osteoblast activity and function under normal and inflammatory conditions. MC3T3-E1 cells were plated at a density of 1×10^5 cells/ml in six-well plates (n=3) and cultured in α-MEM containing 10% FBS, 2 mM L-glutamine and 100 U/L penicillin G and 100 mg/L streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 48 h. After cells reached confluence, the medium was replaced with α-MEM containing 10 mM β-glycerophosphate and 25 μg/ml ascorbic acid to facilitate in vitro mineralization. Cells were treated with 0, 2.5, 5, 10 or 20 μg/ml of dried plum polyphenols for 24 h and then stimulated with 0 or 1 ng/ml of TNF-α, which was the minimum dose needed to significantly inhibit ALP activity and mineralization as determined in preliminary studies. Culture medium, which included TNF-α and the dried plum polyphenol doses described above, was replaced every 3 days. For ALP activity measurements, culture media and cell monolayers were harvested at 7 and 14 days after confluence. For analysis of mineralized nodule formation, cells were fixed at 28 days and stained with alizarin red-S as described below.