Tea polysaccharide inhibits RANKL-induced osteoclastogenesis in RAW264.7 cells and ameliorates ovariectomy-induced osteoporosis in rats

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ABSTRACT

Background and purpose: Tea drinking has positive effects on bone health and may prevent and treat osteoporosis, especially in older and postmenopausal women. Tea polysaccharide (TPS) is a major bioactive constituent in tea. Despite its profound effects on human health, whether TPS has anti-osteoporotic effects remains largely unknown. As such, we investigated the anti-osteoporotic effects of TPS.

Methods: In vitro, TPS effects on osteoclastogenesis were examined using osteoclast precursor RAW264.7 cells. TPS effects on osteoclastogenesis-related expression of marker genes and proteins were determined by gene expression and immunoblotting analyses, respectively. For in vivo studies, 12-week-old female Wistar rats were divided randomly into a sham-operated group (sham) and four ovariectomized (OVX) subgroups: OVX with vehicle (model) and OVX with low-, medium-, and high-dose TPS (0.32, 0.64 and 1.28 g/kg body weight/day, respectively). TPS was administered intragastrically to rats for 13 weeks. Body weight, blood biochemical parameters, organ weight, organ coefficients, femoral length, bone mineral density (BMD), biomechanical properties, and bone microarchitecture were documented.

Results: TPS inhibited osteoclast differentiation significantly and dose-dependently, and its inhibitory effect was not due to toxicity to RAW264.7 cells. TPS suppressed expression of osteoclastogenesis-related marker genes and proteins significantly. In in vivo studies, medium-dose TPS treatment ameliorated OVX-induced calcium loss significantly. Low-dose TPS treatment decreased the activity of acid phosphatase (ACP) in OVX rats significantly. In addition, TPS treatment improved other blood biochemical parameters and femoral biomechanical properties to a certain extent. More importantly, TPS treatment ameliorated bone microarchitecture in OVX rats strikingly because of increased cortical bone thickness and trabecular bone area in the femur.

Conclusion: TPS can inhibit receptor activator nuclear factor-kappa B ligand (RANKL)-induced osteoclastogenesis in RAW264.7 cells and ameliorate ovariectomy-induced osteoporosis in rats.

1. Introduction

With aging populations worldwide, osteoporosis has become a global epidemic that affects millions of individuals, especially post-menopausal women [1,2]. Osteoporosis is a systemic skeletal disorder characterized by reduced bone mineral density (BMD) and deterioration of bone microarchitecture, which increases the risk for bone fragility and fracture [3–5]. The pathophysiology of osteoporosis is very complex, and is potentially caused by genetic disorders, endocrine disorders, and nutritional factors [4]. Currently, many pharmacologic agents are used for the treatment of osteoporosis [6,7]. However, these drugs have evoked adverse effects in patients with osteoporosis. Hence, more specific drugs with fewer adverse effects are needed. It is encouraging that natural products have been shown to be excellent and reliable sources for the development of drugs against osteoporosis [8,9].

Bone remodeling is predominantly a physiologic process regulating bone structure and function, and is achieved through a balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption [10,11]. The imbalance between osteoblasts and osteoclasts contributes to the pathogenesis of osteoporosis [6,10]. Osteoclasts are specialized, large multinucleated cells derived from the monocyte–macrophage hematopoietic lineage. They can develop and adhere to bone matrices, and then secrete acid and lytic enzymes that degrade bone matrices [10,12]. Osteoclastogenesis is regulated by several cytokines, especially receptor activator nuclear factor-kappa B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) [10]. *In vitro*, osteoclast precursors can differentiate into mature osteoclasts under activation of many key transcription factors stimulated by RANKL and M-CSF [12]. Excessive osteoclast activity is closely associated with osteoporosis development. As such, inhibition of osteoclast activity is crucial for the prevention and treatment of osteoporosis.

Numerous studies have demonstrated that tea drinking has positive protective effects on bone mass and may prevent and treat osteoporosis, especially in older women [3,13–16]. Bone homeostasis is maintained by the coordination between bone formation and bone resorption [10]. Tea polyphenols found in green tea can effectively improve bone metabolism by supporting bone formation and suppressing bone resorption [17–23]. Black tea has been shown to have phytoestrogenic effects and enhance bone regeneration in estrogen-deficient rats [23–26]. In addition, black tea may be a prospective adjunct for calcium supplementation to prevent bone loss in a rat model of osteoporosis [27]. Furthermore, we recently demonstrated that water extracts from Pu-erh tea (a type of fermented tea produced in Yunnan Province, China) possesses anti-osteoporotic activity [3]. Studies have revealed that the chemical components contained in the fermented Pu-erh tea are extraordinarily complex [28]. Notably, after fermentation, the caffeine, tea polysaccharide (TPS) and tea pigment levels are increased substantially and are the major components in Pu-erh tea [28,29]. TPS is one of the main bioactive constituents in tea and has been shown to be beneficial for human health [30]. Studies have shown that TPS has excellent antioxidant, anticancer, antidiabetic, anti-obesity, antifatigue, hepatoprotective and immunostimulatory activities [30]. However, whether TPS has anti-osteoporotic effects remains largely unexplored.

To investigate whether TPS could be used as a promising agent in preventing and treating osteoporosis, the present study was designed to evaluate the effects of TPS on osteoclastogenesis using RAW264.7 cells, which is a well-established preosteoclast cell line for the study of osteoclastogenesis. Furthermore, we systematically investigated the pharmacologic effects of TPS on postmenopausal osteoporosis using ovariectomized (OVX) female rats.

2. Materials and methods

2.1. Reagents and antibodies

Escherichia coli-derived recombinant mouse RANKL and rat interleukin (IL)-1β and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and a tartrate-resistant acid phosphatase (TRAP) staining kit were obtained from Sigma–Aldrich (Saint Louis, MO, USA). Antibodies against nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), c-Src, c-Fos, and cathepsin K were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-β-tubulin antibody was sourced from Proteintech (Rosemont, IL, USA) and horseradish peroxidase-conjugated secondary antibody was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ten-percent neutral formaldehyde was obtained from Jinan Biological Technology (Jinan, China). Assay kits for total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), creatinine (CREA), glucose, and alkaline phosphatase (ALP) were purchased from Shenzhen Mindray Bio-Medical Electronics (Shenzhen, China). Acid phosphatase (ACP), calcium, and phosphorus assay kits were sourced from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), BioSino Biotechnology & Science (Beijing, China) and Shanghai Kehua Bio-Engineering (Shanghai, China), respectively. Bone gla protein (BGP) and estradiol (E$_2$) radioimmunoassay kits were purchased from Beijing North Institute of Biological Technology (Beijing, China) and Shenzhen Lalvin Biological Engineering Technology (Shenzhen, China), respectively.

2.2. Preparation of TPS

The TPS-preparation process has been described previously [31]. Briefly, the required amounts of fermented Pu-erh tea were extracted using deionized water and the aqueous extract was collected and concentrated. Then, ethanol was added to the concentrated solution until the percentage of ethanol reached 80%: this process lasted 3 h. The ethanol-sedimentation process was repeated twice. Finally, the sediment was dissolved in deionized water and dried by lyophilization. TPS was dissolved in distilled water, and the solution passed through a 0.2 μm filter (Millipore, Billerica, MA, USA) for further use.

The chemical composition of the prepared TPS was determined according to the standard methods described previously [31–33]. Briefly, the neutral sugar content was determined by the anthrone-sulfuric acid method using glucose as standard. The uronic acid content was measured by the *m*-hydroxydiphenyl method using galacturonic acid as standard. The protein content was analyzed by a modified version of the Bradford method using bovine serum albumin as standard. The caffeine content was determined *via* high-performance liquid chromatography (HPLC). The content of neutral sugar, uronic acid, protein, and caffeine in TPS was 9.94%, 32.63%, 4.51%, and 2.42%, respectively. Notably, there are many chemical components contained in fermented Pu-erh tea, including flavan-3-ols and their derivatives, flavones and their derivatives, other phenolic compounds, alkaloids and their derivatives, and other compounds and trace elements. Three major substances in Pu-erh tea water extract used in our previous study, caffeine, tea saccharide, and polyphenol, account for 4.18%, 9.31%, and 33.13%, respectively [3]. Comparison of the chemical composition of TPS and Pu-erh tea water extract found that TPS used in this study differed from the Pu-erh tea water extract.

2.3. Cell culture

RAW264.7 murine macrophages were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific)