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Anti-osteoporotic effects of an antidepressant tianeptine on ovariectomized rats☆

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ABSTRACT

In the current investigation, the potential alleviating effects of tianeptine against bone loss induced in ovariectomized (OVX) rats was determined. Two weeks following a bilateral ovariectomy operation, tianeptine treatment (12.5 and 25 mg/kg/twice/d) was initiated and continued for twenty-eight consecutive days. Changes in serum and urinary bone turnover biomarkers and osteoclastogenesis-inducing factors were estimated. The femoral bone mineral content was estimated using inductively-coupled-plasma mass spectrometry. Morphometric alterations of distal femoral bones were observed in the cortical and trabecular structures using micro-CT. Finally, femur bones were assessed for histopathological changes. The lack of estrogen significantly increased the levels of bone turnover biomarkers and inflammatory mediators. Mineral concentrations in the femoral bones were reduced in the OVX group. Furthermore, the femoral bone micro-architecture determined using micro-CT and histopathology were significantly altered by estrogen deficiency. Tianeptine, particularly the higher dose, corrected the elevated levels of bone metabolic products and pro-inflammatory cytokines. Tianeptine also improved mineral concentrations in femoral bones and the disturbed morphometric and histopathological features in OVX rats. In conclusion, tianeptine alleviated the osteoporotic changes in OVX animals, which may be via inhibition of the hypothalamic–pituitary–adrenal axis stress and osteoclastogenesis-provoking factors, suggesting attenuation of bone matrix degradation and osteoclast stimulation.

1. Introduction

The prevalence of bone metabolic disease, including osteoporosis, is escalating, affecting 150 million people worldwide, with seventy-five million alone in the USA, Europe and Japan [1–3]. The relationship between depression and bone metabolic disorders has been established in numerous clinical and epidemiological studies [4–6]. Indeed, patients with depression have a greater risk for osteoporotic fractures [7] because they have less bone mass than the non-depressed individuals, particularly at the total femoral output [8–11]. The effect of different antidepressant drugs on bone formation and resorption was also investigated [12–14]. A direct effect of antidepressant drugs on bone mass, particularly those that act on serotonin receptors, is biologically plausible because the expression of serotonin transporter receptors was reported on osteoblasts [15]. Clinical reports revealed the adverse consequences of antidepressant therapy on bone mineral density (BMD) [12,16].

Depression may lead to a persistent stimulation of the stress system of the hypothalamic–pituitary–adrenal axis (HPA), resulting in the production of hypothalamic corticotropin-releasing hormone and, eventually, increased cortisol levels. The impact of hypercortisolemia is the major cause of bone deficit in depression [17]. Furthermore, stress itself is known to provoke osteoclastogenesis and inflammatory markers, such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), which are potent bone resorbing agents [18]. Pro-inflammatory

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mediators trigger the activity and number of circulating osteoclast precursors, promoting osteoclast proliferation to mature bone resorbing cells [19].

Tianeptine is a clinically used antidepressant that protects the hippocampus against stress and the injurious effects of glucocorticoids, suggesting that its antidepressant properties are mediated through an increased rate of neurogenesis [20,21]. Interestingly, tianeptine attenuated stress-associated behavioral deficits in a preclinical depression model and reduced the HPA axis response to stress [21]. Moreover, chronic tianeptine treatment reduces lipopolysaccharide induced inflammatory mediator expression in spleen and plasma [20]. Tianeptine treatment also significantly inhibited periodontal bone loss in olfactory bulbectomized rats and decreased the plasma level of inflammatory cytokines [22]. Therefore, the current investigation was designed to study the potential ameliorative ability of the antidepressant tianeptine against OVX-induced bone metabolic and morphometric changes in Wistar albino rats.

2. Materials and methods

2.1. Drugs and chemicals

Tianeptine (Stablon®) tablets were supplied as a gift from Servier Laboratories, France. Enzyme linked immunosorbent assay (ELISA) kits for bone metabolic parameters, including deoxypyridinoline cross links (DPD), bone specific alkaline phosphatase (BAP), telopeptides of collagen type I (CTX), tartarate resistant acid phosphatase (TRAcP), osteocalcin (OC) and soluble receptor activator of NF-κB ligand (sRANKL), were provided from USCN LIFE, Wuhan EIAab Science Co., Ltd, (Wuhan, China). TNF-α, IL-1β and IL-6 ELISA kits were purchased from R & D systems (Minneapolis, USA). The creatinine diagnostic kit was supplied by RANDOX Laboratories Ltd.

2.2. Experimental animals

In this experiment, thirty-six female Wistar albino rats were used when they were almost three months old. Animals were provided by the Experimental Animals Care Center, College of Pharmacy at King Saud University (KSU). The experimental conditions and euthanasia protocols were consistent with the National Institute of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publications No. 80-23; 1996). Animals were acclimated for 2 weeks before the experiment. They were housed in controlled environments of temperature (21–23° C), humidity (52.5 ± 5%) and 12 h dark/light cycles, and they had free access to Purina rat chow and water *ad libitum* during the acclimation and experimental periods

2.3. Experimental protocol

Twenty-seven rats were subjected to OVX operation under ketamine and xylazine anesthesia. Briefly, the lower inferior area of the rib cage was longitudinally opened on the dorsolateral wall of the body. After exteriorization of the ovaries, the fallopian tubes were ligated and the ovaries were excised. Other sham animals had a similar operational procedure with exception of the ligation and excision steps. To prevent the risk of infection, topical antibiotics were applied to the wounds two times per week. Animals were then allocated in four groups including (A) sham (vehicle) (B) OVX (vehicle), (C) OVX + tianeptine 12.5 mg/kg twice per day PO (OVX + Tia12.5) and (D) OVX + tianeptine 25 mg/kg twice per day PO (OVX + Tia25). The treatments were initiated two weeks after both ovariectomy and sham operations and continued for four consecutive weeks. Weekly body weights were recorded from day 0 until the end of treatment on the same day and approximately same time. At the end of the treatment period, urine samples were collected 24 h before dissection and stored in a freezer at −20 °C until analysis. Blood samples were obtained by cardiac puncture under ketamine and xylazine anesthesia. Samples were then centrifuged for 15 min at 3500 rpm, and the serum was separated and stored in a deep-freezer (SO-LOW Ultra-Low Freezer, Environmental Equipment Cincinnati, OHIO, USA) at −80 °C. Animals were decapitated and the right and left femoral bones were extracted and cleaned of muscle and fat. Uteruses were also removed and cleaned of fat and weighed.

2.4. Determination of bone metabolism

The urinary expression of DPD and the serum levels of BAP, CTX, TRAcP, OC and sRANKL were determined using commercially available ELISA rat kits (USCN LIFE, Wuhan EIAab Science Co., Ltd, Wuhan, China). Urinary DPD expression was reported as the creatinine ratio.

2.5. Determination of inflammatory mediators

Levels of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, were measured in serum using ELISA kits provided by R & D systems (Minneapolis, USA) and the values are expressed as pg/ml.

2.6. Determination of mineral levels in the femoral bone

The left femoral bone of each animal was converted into ashes in a muffle furnace (Lenton thermal designs, Parsons Ln, Hope S33 6RB, United Kingdom) at 650 °C for 42 h [1]. The ashes were then weighed and their weights were correlated with the femoral bone dry weight. A total of 100 mg of each ashed sample was digested in an acidic environment by mixing and overnight incubation with 10 ml of 70% nitric acid in a shaking water bath. The acidic samples were then diluted with deionized distilled water (1:9 ratio) [23]. The diluted samples were then introduced into a ICP-MS (PerkinElmer, Inc. 940 Winter Street, Waltham, MA 02451 USA) to determine the levels of calcium (Ca^{+2}), inorganic phosphorus (P) and magnesium (Mg^{+2}). Femoral bone mineral concentrations are expressed as mg/g ash.

2.7. Determination of bone micro-architecture using micro-CT

The right distal femoral samples were scanned using a SkyScan high-resolution micro-CT (model No. 1172, Bruker, Kontich, Belgium). The scanner was generously provided by the Engineer Abdullah Bugshan Growth Factors Bone Regeneration Chair (GFBR) at KSU. Cortical morphometric parameters, including cortical thickness (CT.th), cortical cross sectional thickness (Ct.Cs.Th), cortical periosteal perimeter (Ct.Pe.Pm), cortical endosteal perimeter (Ct.En.Pm), cortical cross sectional area (Ct.Ar), polar moment of inertia (MMI(p)), eccentricity (Ecc) and cortical porosity (Ct.Po), were determined. Trabecular micro-environment measures included trabecular bone mineral density (BMD), total volume (TV), bone volume (BV), percent bone volume (BV/TV), bone surface (BS), bone surface to volume ratio (BS/BV), bone surface density (BS/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), trabecular pattern factor (Tb.Pf), structure model index (SMI), Euler connectivity (E.Con), euler connectivity density (E.Con.D) and degree of anisotropy (DA). Briefly, each femoral bone sample was positioned such that its longitudinal axis was parallel to the tubular container of the device. Scanning conditions were as follows: 70 kv, one mm