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Do acid suppressive drugs (pantoprazole and ranitidine) attenuate the protective effect of alendronate in estrogen-deficient osteoporotic rats?

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ABSTRACT

Background: Alendronate is a drug of choice in the treatment of postmenopausal osteoporosis. Acid suppressive drugs are recently used in combination with alendronate to protect against stomach ulceration and oesophagitis occurring as serious problems in patients under this treatment.

Aim of the work: To study the effect of pantoprazole and ranitidine on the anti-resorptive effect of alendronate in a rat model of osteoporosis.

Material and methods: 64 female Wister albino rats were included (8 rats/group). Treated groups were Ovariectomized bilaterally to induce osteoporosis. Rats were treated orally with alendronate (3 mg/kg/day), ranitidine (20 mg/kg/day), pantoprazole (3 mg/kg/day), combined alendronate and ranitidine or alendronate and pantoprazole for 4 weeks. Bone mineral density (BMD) was determined using dual-energy X-ray absorptiometry scan. Serum bone-specific alkaline phosphatase (BSALP), serum calcium, cortical thickness and histopathological examination were assessed.

Results: Induction of osteoporosis significantly elevated serum BSALP, decreased serum calcium, bone mineral density and cortical thickness and significantly increased the histopathological score. Ranitidine treatment resulted in insignificant changes in tested parameters. Pantoprazole given alone, significantly decreased serum calcium and increased serum BSALP. At the end of the experiment, alendronate significantly improved BSALP level, serum calcium, BMD, cortical thickness and histopathological score. This improvement was not affected by combining ranitidine with alendronate. Meanwhile, pantoprazole with alendronate caused a significant deterioration of tested parameters.

Conclusion: Ranitidine proved safer than pantoprazole for patients with high risk of osteoporosis and in alendronate-treated patients. Clinicians should carefully judge the indication of use of acid suppressing medications in osteoporotic patients.

1. Introduction

Osteoporosis is a common disease that is characterized by massive bony resorption, decrease in its mass and disruption of its architecture predisposing to fractures. Osteoporosis is a common worldwide bony disorder, particularly in elderly. Near half of the women and about 14% of the men above the 50th are at risk of fracture due to osteoporosis [1]. Estrogen deficiency is one of the main factors causing osteoporosis. Fat mass was proved also to play role in bone metabolism in postmenopausal women [2]. Other causes are due to either calcium or vitamin D deficiencies or secondary hyperparathyroidism. But, because of its preventable and curable nature and awareness of almost 75% of women with this fact, easy diagnosis and compliance to treatment became successful [3].

Oral bisphosphonates (BPs) are considered as the first-line therapy to prevent fractures in osteoporotic patients. Bisphosphonates are classified into non-nitrogen containing BPs as etidronate and the more potent, nitrogen-containing BPs as alendronate [4]. Two distinct mechanisms of action have been revealed. The non-nitrogen containing BPs interfere with bone resorption by producing cytotoxic ATP analogues which prevents the mitochondrion from functioning properly and cause osteoclastic apoptosis [5]. In contrast, the nitrogen-containing BPs inhibit key enzymes regulating the mevalonic acid pathway, interfering with small GTPases needed for bone-resorption by osteoclasts. The most commonly reported adverse side effects due to the intake of BPs are ulceration and oesophagitis. Therefore, acid suppressive medications (ASM) are usually prescribed with BPs [6].

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Proton pump inhibitors (PPIs) and histamine 2 receptor antagonists (H_2RAs) are frequently used ASMs, taken on long-term basis [7]. H_2RAs inhibit acid secretion stimulated by gastrin or pentagastrin. They are highly effective drugs for various diseases as Zollinger-Ellison syndrome and peptic ulcer [8]. Ranitidine is a selective potent competitive H_2RA with fewer side effects [9]. PPIs target the H+/K+ ATPase of gastric parietal cells and inhibit the final step in HCl release [10]. PPIs reduce gastric acid secretion by nearly 98% due to irreversible inhibitory effect on the proton pump in gastric parietal cells [11]. Conditions that cause hypo/achlorhydria as gastrectomy, pernicious anemia and atrophic gastritis are associated with increased occurrence of osteoporosis [12]. Inhibition of secretion of hydrochloric acid by use of ASMs especially on long basis increase the pH of the stomach or small intestine which may inhibit absorption of insoluble calcium and affect bone mineralization [13].

The aim of the present study was to investigate whether the combination of either of ranitidine or pantoprazole with alendronate attenuates its antiresorptive effect or not in estrogen-deficient osteoporotic rats.

2. Materials and methods

Sixty-four female Wistar albino rats, weighing 150–200 mg matched for age and weight, bred in the animal house of the Research Institute of Ophthalmology, were used in this study. They were exposed to good ventilation and bred at ambient room temperature (21 ± 2 °C), and relative humidity (65–70%), fed with laboratory diet and given water and diet as needed. Before beginning the experiment they were left for a whole week to accommodate with the new environment. The study protocol was approved by the Institutional Reviewer Board of Faculty of Medicine, Cairo University and the animal experiments were carried out in accordance with the ethical guidelines of animal welfare.

The drugs and chemicals used included pantoprazole (Altana Pharma AG, Egypt). ranitidine (Lek Pharma, Slovenia) and alendronate sodium trihydrate (Sigma-Aldrich company, Germany). Drugs were freshly prepared and dissolved in tap water and administered daily for 28 days. Kits for colorimetric estimation of bone specific alkaline phosphatase in serum were used (Leader trade company).

2.1. Experimental design

Rats were divided into eight groups (8 rats each): (1) Group I (control non-ovariectomized rats; NOVX): Rats of this group received 1 ml distilled water orally for 8 weeks and served as a control group; (2) Group II (Sham-operated group): Rats of this group were subjected to two horizontal incisions beneath the costal margin in the right and left flanks and the incision was closed without ovariectomy and left with no treatment for 28 days. Then received 1 ml distilled water for another 28 days; (3) Group III (ovariectomized 'OVX', non-treated group): bilateral ovariectomy was done to rats of this group according to *Pytlik* et al. [14] and left with no treatment for 28 days. Then received 1 ml distilled water for the following 28 days; (4) Group IV (OVX, ranitidine-treated group): as in group III then treated with ranitidine orally in a dose of 20 mg/kg [15] for another 28 days; (5) Group V: (OVX, pantoprazole-treated group): as in group III and then received pantoprazole orally in a dose of 3 mg/kg for 28 days [16]; (6) Group VI: (OVX, alendronate-treated group): as in group III and then treated with alendronate orally in a dose of 3 mg/kg [17] for another 28 days; (7) Group VII: (OVX, alendronate and ranitidine-treated group): as in group III and then treated with alendronate orally in a dose of 3 mg/kg and ranitidine orally in a dose of 20 mg/kg for another 28 days; (8) Group VIII: (OVX, alendronate and pantoprazole-treated group): as in group III then began alendronate treatment orally in a dose of 3 mg/kg and pantoprazole orally in a dose of 3 mg/kg for 28 days.

For induction of osteoporosis, rats of groups III to VIII were anaesthetized by intramuscular injection of 46 mg/kg ketamine [18] and two horizontal incisions were made beneath the costal margin in the right and left flanks. The ovaries were exteriorized, ligated and excised then the abdomen was closed [14].

2.2. Biochemical tests

Collection of blood from the rats' tail vein was done before the experiment and repeated every two weeks throughout. Blood samples were incubated at 37 °C until blood clotted. Separation of serum was done by centrifugation. Then the following parameters were measured in serum:

2.2.1. Serum bone-specific alkaline phosphatase (BSALP) level

The reagent was prepared by reconstitution of 10 ml of the substrate with 10 ml of the buffer. A volume of serum (0.02 ml) was mixed with 1 ml of reagent at 37 °C. Absorbance (A) was read initially, after 1, 2 and 3 min. The alkaline phosphatase (ALP) activity was calculated using the following formula: ALP(U/L) = 2760 × ^A at 405 nm/min [19]. The bone fraction was inactivated by heat. Therefore, the heat stable activity was determined using serum that has been heated in a thin walled glass tube at 56 °C ± 2 °C for exactly 10 min and then cooled in iced water. By subtracting the results that were obtained from the total ALP level, we determine the bone specific isoenzyme [20].

2.2.2. Serum calcium level

The concentrations of serum calcium were determined spectrophotometrically using an automated analyzer (Hitachi 7070; Japan) as described by *Gitelman* [21] and *Goodwin* [22].

2.3. Bone mineral density (BMD) measurement

Dual energy X-ray absorptiometry (DEXA) is the clinical method used to evaluate bone quality. The BMD of one femur in each animal was measured by DEXA (Norland XR-46, Norland/Swiss ray, USA) at a scan pitch of 1.5 mm and a scan speed of 60 mm/s. The animals were scanned 4,6 and 8 weeks from the start of the experiment.

2.4. Bone histomorphometry

At the end of the experiment, animals were sacrificed. One of the hind limbs was separated and the femur was fixed in 4% formalin solution. It was decalcified, embedded in paraffin, cut and then stained with Hematoxylin- Eosin (H&E). The cortical and trabecular bone thickness area in the proximal femur metaphysis was measured by quantitative image analysis system (Leica Qwin 500 image analyzer computer system) [23]. Histopathological scoring system included thickness of cortical bone, presence of fractures, bone defects within thickness, homogenous appearance of matrix, arrangement of Haversian systems and periosteal regularity. Each parameter was assessed and given a score. Score 0: defect up to <25%, score 1: defect 25–50%, score 2: 50–75% and score 3: >75% [24].

2.5. Statistical analysis

Data was coded and entered using the statistical package SPSS version 24. Data was summarized using mean and standard deviation (SD) for quantitative variables. Comparisons between groups

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