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Research Article

Efficacy and side effects of different regimens of experimental postmenopausal osteoporosis therapy with zoledronic acid and their correction by combination with resveratrol

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Abstract

Introduction: As an age-associated metabolic disease of the skeleton, osteoporosis (OP) causes a significant medical and socio-economic burden. One of the challenges in clinical practice is the adverse effects of widely used bisphosphonates. Therefore, the search for preventive pharmacotherapeutic strategies is needed.

Materials and Methods: Sixty female Wistar rats randomized into 6 equal groups were used to study the effects of a single and prolonged intravenous administration of zoledronic acid (ZA) without and in combination with an endothelioprotective dose of resveratrol (2 mg/kg intraperitoneally daily), compared with sham-operated and osteoporotic animals. X-ray densitometry and histological methods were used.

Results and Discussion: Already at 4 weeks after bilateral ovariectomy (OVx), the typical signs of osteoporotic lesions comparable with OP in humans are present. A single administration of ZA following 4 weeks of OP had a significant corrective effect on bone structure and the fatty component of bone marrow. After a second dose of ZA at 8 weeks after OVx, signs of bone damage were detected by week 12. The addition of 4 weeks of resveratrol therapy after the second dose of ZA (until 12 weeks after OVx) leads to switching of bone marrow precursors to osteoblastic differentiation, reducing the adipocytic component; cytoprotective action on osteocytes with their switching to a synthetic phenotype, reducing the potential activation of osteoclasts and blocking periosteocytic osteolysis; and protection of bone vessels via the endothelioprotective properties of resveratrol.

Conclusion: An endothelioprotective dose of resveratrol exerts multitarget cell- and endothelium-dependent mechanisms of protective action when combined with prolonged zoledronic acid therapy, preventing its adverse effects.

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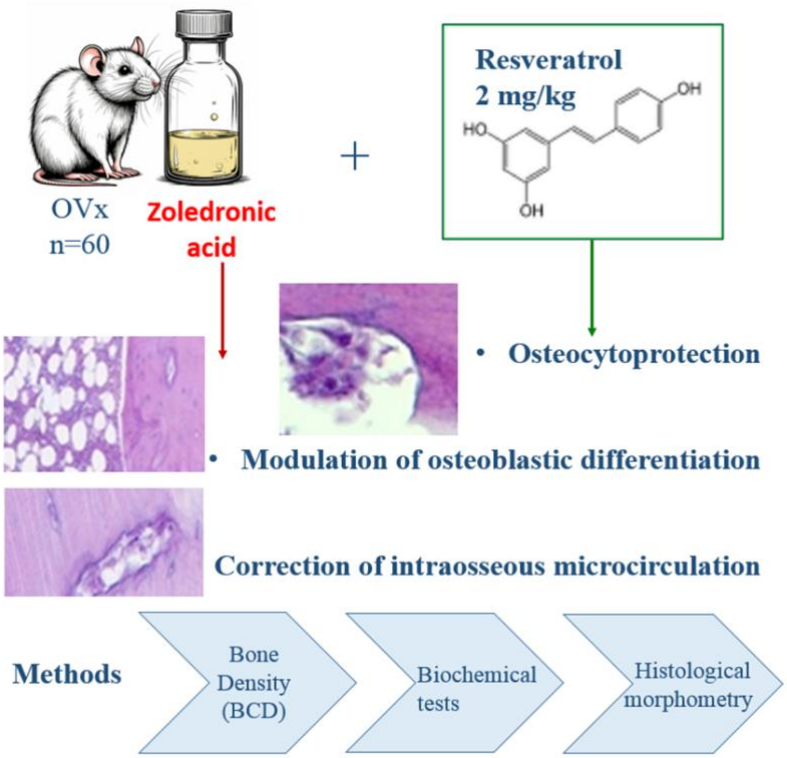
Graphical Abstract



Keywords

osteoporosis; zoledronic acid; resveratrol

Introduction

Osteoporosis (OP) is one of the main metabolic and age-associated diseases of the skeleton. Epidemiological data in different countries are similar, as there are no geographic regions or ethnicities free from this problem. According to the latest data (Rozhinskaya and Lutsenko 2021), due to significant aging of the population, by 2035 a substantial increase in the frequency of hip fractures is expected (based on the calculated probability, this fracture will occur every 3 minutes). By 2050, a one-third increase in the number of osteoporosis patients is expected in Russia.

Among the forms of osteoporosis, primary osteoporosis, which is not associated with other pathologies or the effects of medications, predominates. It accounts for 95% of osteoporosis in postmenopausal women and 80% of osteoporosis in men over 50 years old. Being an age-associated disease, osteoporosis represents one of the key gerontological problems.

In addition to the issue of preventing and treating osteoporosis, there is the problem of complications caused by medications, with bisphosphonates being first-line agents, having been introduced into clinical practice in 1995. After 10 years of their use, reports of their serious side effects began to appear, the most severe of which are atypical femoral fractures (Odvina et al. 2005) and osteonecrosis of the jaw (Spevak and Tsymbal 2017; Marx 2003).

Attention to the seriousness of the factor of cautious attitude and low patient adherence to treatment due to fear of complications and uncomfortable pharmacotherapy regimens was already noted in the earliest guidelines on metabolic bone disease (Camacho, 2019). This determines the relevance of studying pharmacologically active substances for the prevention of complications of basic therapy – a kind of 'covering' drugs. A possible drug of this kind is the plant polyphenol resveratrol, possessing a wide spectrum of biological activity including regulation of cellular proliferation and differentiation, endothelioprotection and cytoprotection (Vasiliev et al. 2007).

The aim of this study is to investigate the structural and functional changes of the femur and tibia with the combination of prolonged zoledronic acid therapy and resveratrol.

Materials and Methods

Experimental animals

The experiment was performed on 60 female Wistar rats. The ethical principles for the handling of laboratory animals were observed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, ETS No. 170. All painful procedures performed on animals were carried out in compliance with the relevant regulatory standards: Directive 2010/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes; and the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). The study was approved by the Animal Ethics Committee of Belgorod State National Research University (BelSU), Expert Opinion No. 02-01i/25 dated 09.01.2025.

Experimental design

The experimental animals were randomized into 6 equal groups:
1. False-operated (FO, intact control);
2. 4 weeks after ovariectomy (OVx4);
3. 8 weeks after ovariectomy (OVx8);
4. OP model by bilateral ovariectomy and single intravenous administration of zoledronic acid in a dose of 0.006 mg per 100 g of the animal's body weight, 3 days after 4 weeks of osteoporosis, with bone material examined on the 8th week after OVx (OVx+ZA);
5. OP model by bilateral ovariectomy (OVx) and two-time intravenous administration of zoledronic acid on the 4th and 8th weeks after ovariectomy and observation until the 12th week (OVx+ZA2);
6. OP model by bilateral ovariectomy (OVx) with two intravenous doses of zoledronic acid at weeks 4 and 8 post-OVx, followed by daily intraperitoneal resveratrol (2 mg/kg) starting after the second ZA injection for up to week 12 (OVx+ZA2+Res).

Compounds under study

Zoledronic acid was selected from the available bisphosphonates for the study as a representative of third-generation nitrogen-containing drugs, which have the greatest effect (Knyazkova 2014; Cremers et al. 2019). The working solution was prepared in saline from a lyophilizate for intravenous infusion concentrate (RUE "Belmedpreparaty", Republic of Belarus) and administered into the tail vein at a dose equivalent to a single 4 mg administration for a human, based on a 70 kg body weight, which amounted to 0.006 mg per 100 g of the animal's body weight. Resveratrol (Sigma-Aldrich, USA) was administered intraperitoneally in saline at a known endothelioprotective dose of 2 mg/kg of body weight.

Methods of study

At the end of the experiments in all series, before euthanasia, the animals under chloral hydrate anesthesia were subjected to densitometry of the studied parts of the skeleton using a multifunctional laboratory X-ray system IN-VIVO MS FX PRO (Bruker; USA) with a molecular imaging system and licensed software – BoneDensity Software, to assess bone mineral density by the BCD (Bone Column Density) index. After this, the anesthetized animals were removed from the experiment by cervical dislocation. For histological examination, the proximal halves of the femur from the head to the middle of the diaphysis were extracted by exarticulation from the hip joint.

Bones were decalcified in the 'Surgipath Decalcifier II' solution (Leica, Germany) according to the recommended protocol. The decalcified material was automatically embedded in paraffin, followed by staining of 7 μm thick histological sections with hematoxylin and eosin, according to Mallory, and according to Van Gieson.

For examination of histological specimens and morphometry, their digital counterparts were created using the Hamamatsu NanoZoomer-SQ Digital Slide Scanner (Japan) image scanning and archiving system, and the NDP.View 2 analytical module, which was used to measure the linear parameters of bone structures and to produce illustrations. The specific areas of bone structures and bone marrow spaces were assessed using the point-counting method according to G.G. Avtandilov, using a computer analog of a transparent morphometric grid with 50 nodal points projected onto the images.

Biochemically, the levels of total calcium and alkaline phosphatase in peripheral blood were evaluated (the study was carried out in the ArtVet Network Laboratory, Russia).

Statistical analysis

Quantitative data were recorded and analyzed using MS Excel tables. To determine the statistical significance of differences, taking into account the normality of the distribution of traits, Student's t-test was used.

Results

Histostructural, osteodensitometric, and specific biochemical indicators suggest that already 4 weeks after bilateral ovariectomy there are signs that can be extrapolated to osteoporotic bone lesions in humans. At this stage of the experiment, the spectrum of bone lesions specific to human osteoporosis was revealed: thinning of the bone trabeculae (Fig. 1), an increase in the size of the intertrabecular spaces, and a decrease in bone mineral density. Structural loss of bone trabeculae was confirmed as a significant ($p < 0.01$) decrease in their average thickness to 47.1 ± 0.9 μm compared with 57.4 ± 1.4 μm in sham-operated animals. The structures of the lacunocanalicular system (LCS) changed significantly. Considering the predominantly elongated ellipsoid shape of the lacunae, their average major (longitudinal) size was morphometrically determined, and a significant ($p = 0.003$) increase to 11.5 ± 0.2 μm was found (in sham-operated animals: 8.9 ± 0.8 μm). Histologically also, at this stage of the experiment, disruption of microarchitecture was revealed, with rarefaction of the trabecular structure, an increase in the proportion of bone marrow spaces with an increase in their fat component, basophilic homogeneous foci of chondroid degeneration, lamellar splitting of bone substance, and the most specific sign – microfractures of the bone trabeculae. Frequently, fragments of osteonal structures with deformed remnants of capillary walls inside were detected. The inner contour of the lacunae lost clarity, and the space between it and the osteocytes located inside appeared as a weakly basophilic lysed mass. Osteocytes with pyknotic, deformed contours were shifted to one side of the lacuna; the radial pattern of their processes and canaliculi in the perilacunar areas was erased or represented by short strokes.

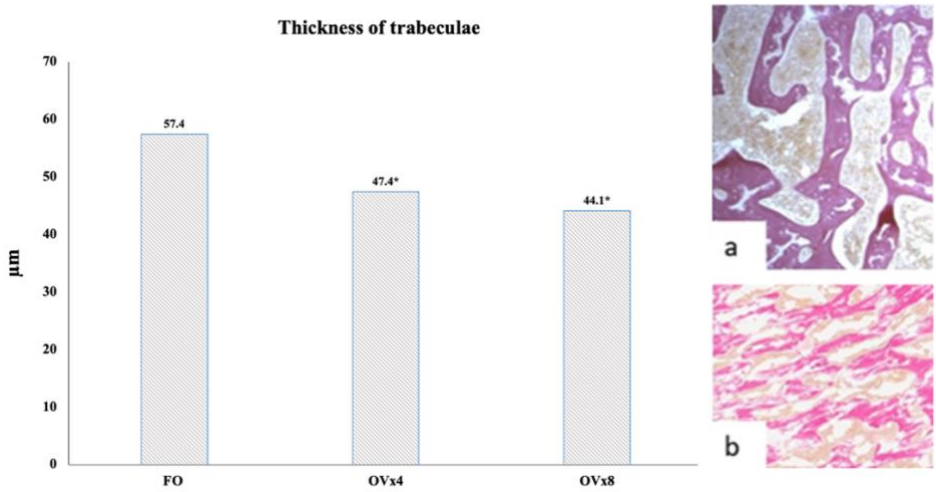


Figure 1. Changes in the proximal femur 4 weeks after bilateral ovariectomy: (**a**) rarefaction, decreased orderliness of microarchitecture, thinning of trabeculae, expansion of bone marrow spaces; (**b**) chaotic distribution and fragmentation of bone trabeculae. Staining according to Van Gieson. Scanned images. Viewing magnification: ×10 (a), ×40 (b). FO – false-operated animals, OVx4 – 4 weeks after ovariectomy, OVx8 – 8weeks after ovariectomy; * – reliable differences ($p<0.05$) from FO.

The osteoporotic nature of the described histopathological changes was confirmed by the results of osteodensitometry. At this stage of the experiment, the BCD index decreased significantly (by 75%) with $p < 0.01$. The severity of osteoresorptive processes is confirmed by both the detection of resorption-active osteoclasts on the trabecular surfaces (Fig. 2) and the results of biochemical studies. Total blood calcium levels at this stage of the experiment significantly ($p < 0.05$) increased from 2.24 ± 0.04 to 2.47 ± 0.03 mmol/L. At the same time, alkaline phosphatase levels significantly ($p = 0.03$) decreased from 73.1 ± 0.9 U/L to 64.6 ± 0.8 U/L.

In 8 weeks after bilateral ovariectomy, progression of the above-described pathohistological and osteodensitometric changes was detected. As in the previous series, these included the full spectrum of bone lesions specific to osteoporosis and were associated with more pronounced osteoclastic activity. Colocalization of resorption-active osteoclasts and lymphohistiocytic infiltrates was frequently detected. Changes in osteocytes and the lacunar-tubular system

persisted and became more pronounced (Fig. 3). The average size of osteocyte lacunae (11.2 ± 0.4 μm) remained at the same level as in the previous period, also significantly exceeding that in sham-operated animals. Osteocytes in dilated lacunae show signs of apoptotic changes: pronounced pycnomorphic nuclear changes with margination of heterochromatin clumps, shifted toward the periphery of the lacunae. Many small lacunae are empty. The extralacunar portions of the processes are short, with an "amputated" appearance, resulting in radial striations in the interlacunar spaces, corresponding to bony canals with osteocyte processes. Emptying of the lacunae is common.

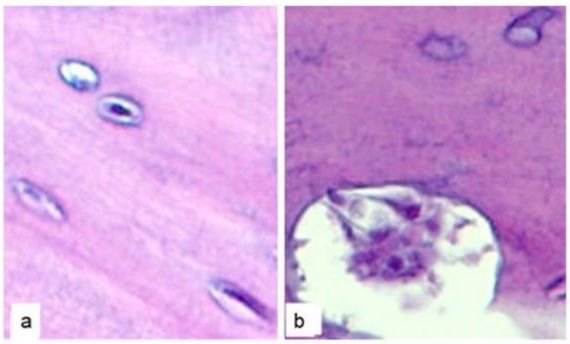


Figure 2. Changes in lacunae with osteocytes (**a**) and a resorptively active osteoclast (**b**) 4 weeks after bilateral ovariectomy: (**a**) pyknosis, peripheral displacement of osteocytes with widening of the lacunae; (**b**) dystrophically altered osteocytes in the thickness of the bone trabecula, a resorption lacuna with an active osteoclast on its surface. Hematoxylin and eosin staining. Scanned specimens. Viewing magnification: ×40.

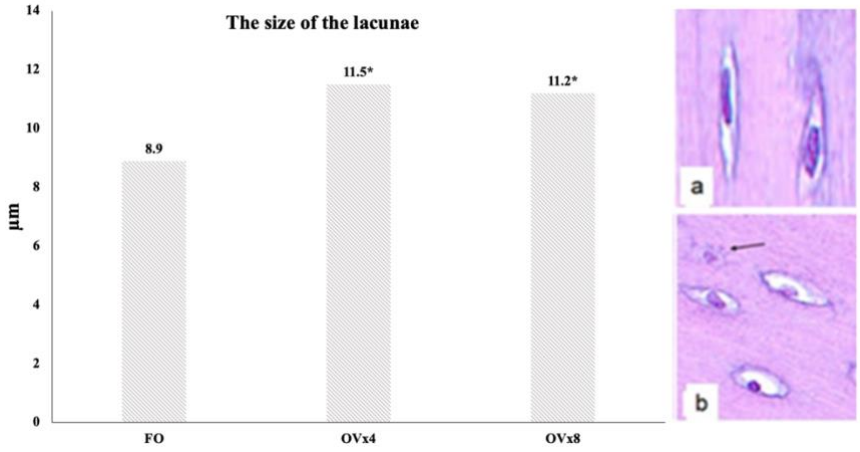


Figure 3. Changes in lacunae and osteocytes 8 weeks after ovariectomy: (**a**, **b**) widening of lacunae, displacement of pycnomorphic osteocytes to their periphery, extralacunar areas of the processes of the "amputated" type (arrow). Staining with hematoxylin and eosin (**a**, **b**). Scanned specimens. FO – false-operated animals, OVx4 – 4 weeks after ovariectomy, OVx8 – 8weeks after ovariectomy; * – reliable differences ($p<0.05$).

As in the previous series, osteodensitometry results confirmed the osteoporotic nature of the bone changes. Bone mineral density remained significantly by 17% lower ($p = 0.02$), decreased compared to sham-operated animals. The increase compared to 4-week osteoporosis was insignificant ($p = 0.06$) and is likely related to the presence of focal signs of bone regeneration at this time, in the form of superposition of bone plates on pre-existing ones, reflecting a compensatory response of intact bone areas.

A single administration of zoledronic acid following 4 weeks of osteoporosis had a significant corrective effect on bone structure and the fatty component of bone marrow. In experimental group, which received a single administration of the drug 3 days after the onset of 4 weeks of osteoporosis, the overall microarchitecture of the trabecular bone of the head by 8 weeks (Fig. 4) was virtually identical to that in sham-operated animals and was represented by orderly arranged anastomosing beams, relatively narrow bone marrow spaces occupying up to a third of the section area and containing a moderate number of adipocytes. The beams had an ordered lamellar structure with tightly adherent plates and thin cementing lines between them. Despite the observed significant 50% increase in the average longitudinal size of the lacunae (12.4 ± 0.6 μm), they contained osteocytes with an intact light microscopic structure, and the

interlacunar areas had a radial pattern of their processes in the form of short continuous lines or dotted lines, which is due to their tortuosity and multiple occurrences in the section. A positive effect is confirmed by the results of trabecular morphometry, the thickness of which (57.3 ± 1.0 μm) did not differ significantly from that in sham-operated animals.

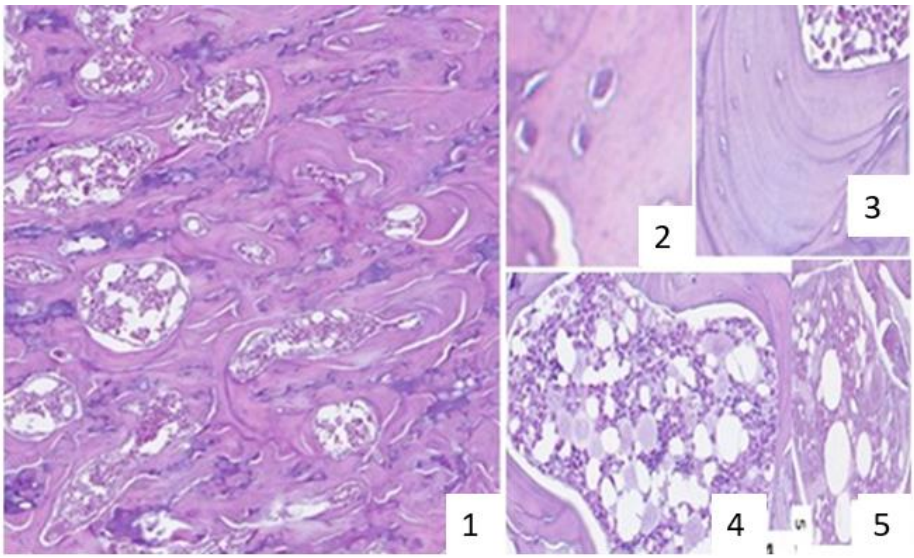


Figure 4. Histostructural changes in the femoral head 8 weeks after a single administration of zoledronic acid in 4-week osteoporosis (group 4): **1** – compact overall architecture of bone trabeculae, narrow bone marrow spaces with a small volume of fat component; **2** – intact osteocytes in lacunae; **3** – trabeculae with a clear lamellar structure, preserved osteocytes; **4** – picture of bone marrow lipomatosis from thegroup with 8-week osteoporosis for comparison with group 4 (5), showing a significantly smaller number of adipocytes. Hematoxylin and eosin staining. Scanned specimens.

After two doses of zoledronic acid, significant structural changes were histologically detected, indicating bone damage. Although the thickness of bone trabeculae (Fig. 5) remained greater than in untreated OP animals, and the reduction compared to sham-operated animals and single-dose therapy was insignificant, their intercellular matrix underwent significant local changes, revealing signs of bone matrix disorganization (Fig. 6).

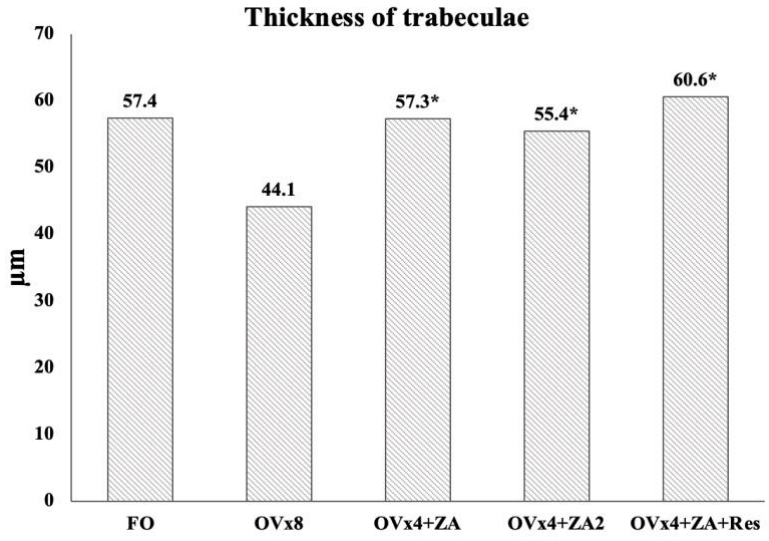


Figure 5. Changes in the thickness (μm) of the trabeculae of the femoral head after 8 weeks of single administration of zoledronic acid (OV4+ZA), after 12 weeks of double administration without resveratrol (OV4+ZA2), and with resveratrol (OV4+ZA2+Res). * – reliable differences ($p<0.05$) from OVx8.

Microfractures of the trabeculae were detected, topographically associated with pronounced bone marrow lipomatosis (Fig. 7). Osteocytes and the lacunar-tubular system were significantly altered. Osteocytes in many lacunae were arranged asymmetrically, with pycnomorphic nuclei and homogenized cytoplasm; the pattern of their processes was erased. A light-colored space –

a halo – formed around many cells. Morphometry of the lacunae revealed a significantly ($p < 0.001$) increased mean longitudinal diameter, at the same level as after a single injection. Furthermore, the dilated lacunae may be associated with osteocyte death, not just periosteocytic osteolysis. The characterized changes in the intercellular substance are highly likely to form the basis for the detected decrease in bone mineral density, which, in comparison with sham-operated animals, with a single administration significantly decreased by 37%, and with double administration the relative decrease reached 40%. Changes were detected in the periosteal, intraosseous, and bone marrow microvessels, indicating predominant damage to the endothelial lining, with subsequent spread throughout the entire wall thickness, which were naturally accompanied by hemorheological disturbances. The internal contour of the walls had a split appearance due to detachment of sections of the endothelium, while the remaining wall thickness had a homogenized appearance. Remnants of damaged intraosseous vessels were detected. The number of intraosseous sinusoidal vessels was reduced. Hemorheological abnormalities included widespread intraluminal and parietal erythrocyte aggregates and hyaline microthrombi.

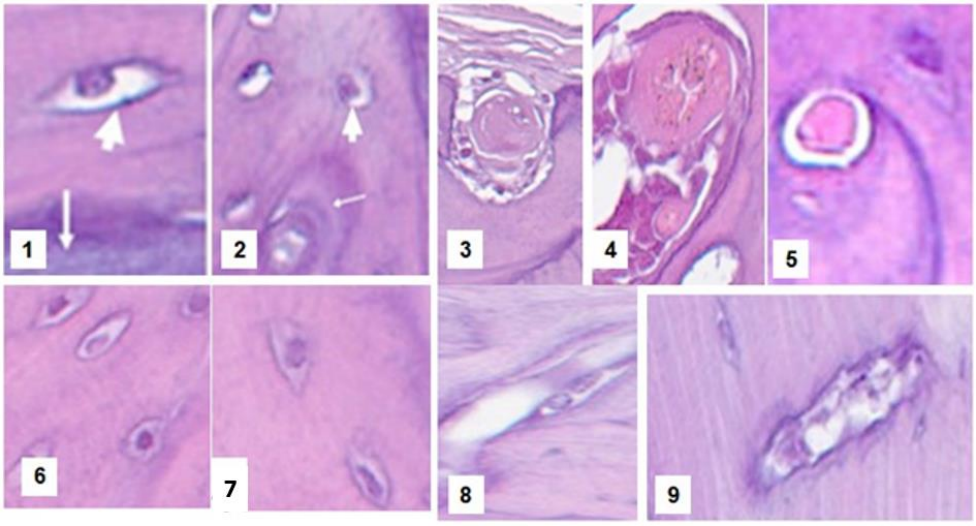


Figure 6. Bone (1, 2, 6, 7) and vascular (3-5, 8, 9) changes in the femoral head after 12 weeks of monotherapy with double administration of zoledronic acid (1-5) and in combination with resveratrol (6-9): **1** – osteocyte apoptosis and lacuna dilation (above the arrow), chondroid degeneration of the intercellular substance (below the arrow); **2** – osteon remnant, apoptotic osteocyte; **3** – sclerosis of small periosteal arteries; **4** – plasma infiltration of the bone marrow artery wall; **5** – hyaline thrombus in the residual lumen of the capillary; **6**, **7** – intact osteocytes; unchanged intraosseous venule (**8**) and capillary (**9**). Hematoxylin and eosin staining. Scanned specimens.

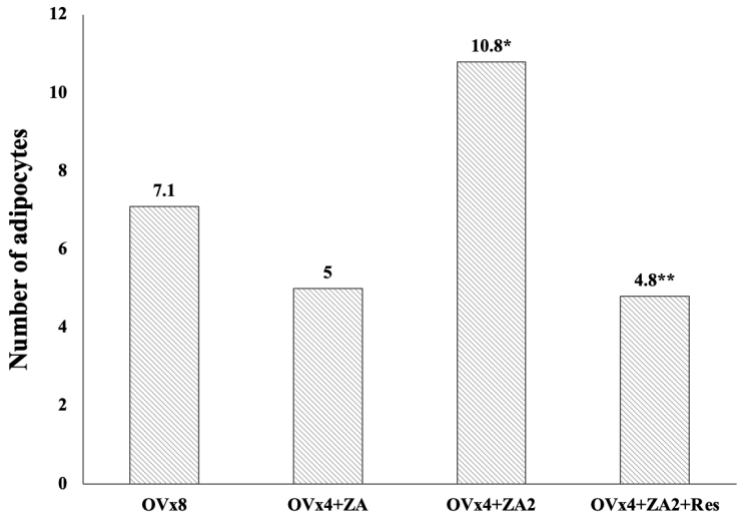


Figure 7. Average number of adipocytes in the bone marrow space. Femoral head changes at week 12 after double zoledronic acid administration and its combination with resveratrol. OVx8 – 8 weeks after ovariectomy; ZA – zoledronic acid, Res – resveratrol. * – reliable differences ($p<0.05$)

A combination of prolonged (two-dose) zoledronic acid and resveratrol therapy from the second administration (8 weeks after ovariectomy) to 12 weeks revealed significant corrective effects. These effects included cytoprotection of osteocytes and the lacunar system, with a reduction in the number of damaged cells and empty lacunae, and an increase in the number of moderately changed and unchanged cells.

With a combination of double administration of zoledronic acid and 4-week therapy with resveratrol, a shift in adiposity to the hypertrophic type was revealed (Fig. 8). With pronounced size polymorphism, adipocytes exceeding the average in diameter were frequent, and their size was significantly larger (50.1 ± 0.9 μm) compared with those in both sham-operated animals (39.3 ± 0.8 μm) and the group without resveratrol ($p < 0.001$ for both comparisons). However, assessment of the average number of adipocytes per intertrabecular space revealed a significant ($p = 0.02$) decrease (4.8 ± 0.8) compared with the group without resveratrol (7.2 ± 0.6).

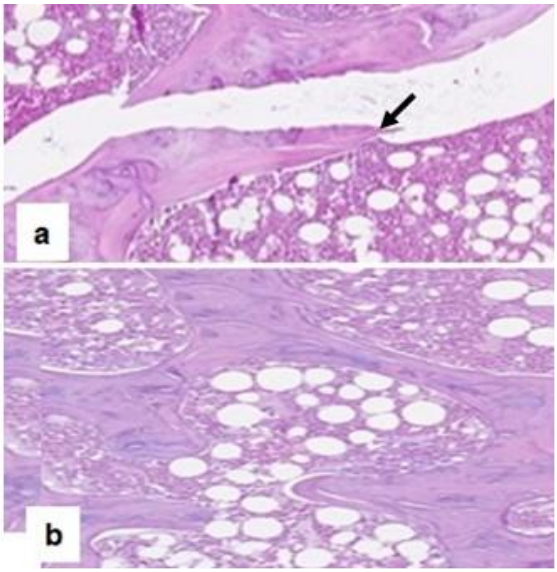


Figure 8. Changes in the femoral head by week 12 after double administration of zoledronic acid (**a**) and its combination with resveratrol (**b**): a – colocalization of a marginal trabecular microfracture with bone marrow lipomatosis; **b** – mixed bone marrow lipomatosis and a reduction in the absolute number of adipocytes in the intertrabecular spaces. Hematoxylin and eosin staining. Scanned specimens.

Discussion

Literary data show that the question of the effects and mechanisms of zoledronic acid (ZA) in the pharmacotherapy of osteoporotic lesions, despite a large literature base, is not without interpretative complexities and contradictions.

Based on the results of our own study, we first note the complete suppression of osteoclastic resorption by zoledronic acid (ZA). Therefore, it is likely that with a single administration starting after 4 weeks (i.e., at 8 weeks after ovariectomy), when complete osteoporosis develops without therapy, ZA arrests the progression of bone changes. However, with two administrations over a total period of 12 weeks after ovariectomy, adverse effects of the drug are evident. From the point of view of bone remodeling biology, the negative aspects of bisphosphonate therapy may have the following theoretical explanation.

First, osteoclasts in the initial phase of bone remodeling (initiation) ensure the removal of functionally unsuitable (aged, damaged) bone structures from areas subject to renewal, without affecting normal bone. Because bisphosphonates, especially those of the latest generation, are potent inhibitors of osteoclast formation and activity, this important natural process is inhibited or reduced. The result is the accumulation of unremoved bone structures with mechanical "fatigue" and microdamage, leading to deterioration of the bone's physical properties.

Secondly, in addition to the primary osteoresorptive function, a complex system of direct and bone matrix-mediated interactions exists between osteoclasts, osteoblasts, and their precursors (Daponte et al. 2024), and osteoclasts regulate the formation and functioning of osteoblasts and osteocytes. Therefore, the exclusion of osteoclasts from the bone cell system leads to impaired osteogenesis.

Therefore, the following existing recommendations for optimizing the treatment of bone and joint pathology with bisphosphonates in the context of hypoestrogenism are logical (Strukov et

al. 2022): modern osteoporosis therapy should take into account the alternation of the resorption and mineralization phases, that is, it should not interfere with the natural functioning of the osteoclast-osteoblast-osteocyte system. In this regard, the use of courses of osteoprotective therapy is advisable.

Experimental data, though sparse compared to clinical data, support this position. Earlier, Hirano et al. (2000) hypothesized that since one of the goals of remodeling – i.e., the physiological regeneration of bone tissue – is the elimination of microdamage, suppression of the resorption phase worsens bone quality due to the accumulation of microdamage. Based on this, a study was conducted to examine the effects of high doses of etidronate on bone mechanical properties and microdamage accumulation. Mature dogs aged 1–2 years were administered the drug at a dose of 0.5 mg/kg or 5.0 mg/kg per day for 1 year. Radiographs were taken at the beginning of the study and monthly for 7–12 months. After bone harvesting, biomechanical testing, histomorphometry, and microdamage analysis were performed. Rib and/or spinous process fractures were found in 10 of 11 dogs receiving the higher dose of etidronate. Only one spinous process fracture was found in dogs receiving the lower dose, and no fractures were found in the control group. Biomechanical testing revealed decreased mechanical strength of the ribs and lumbar spine.

However, subsequent data suggest that zoledronic acid stimulates osteogenesis by counteracting the effects of oxidative stress (Jin et al. 2020). This indicates that this issue remains controversial and that further research is needed. In addition to changes in bone structure, prolonged zoledronic acid therapy significantly alters intraosseous vessels. Qualitatively, this is manifested by damage to the endothelial lining of intraosseous capillaries and sinusoids, and quantitatively, by a significant reduction in their number per bone volume. Furthermore, sclerotic changes in the walls of periosteal and bone marrow small arteries and arterioles have been detected, along with plasma infiltration, a precursor to angiosclerosis. To explain the changes caused by zoledronic acid therapy, it is appropriate to draw on knowledge about another serious complication of bisphosphonate therapy – osteonecrosis of the jaws (ONJ) – first described in 2003 as an isolated clinical case (Marx 2003) and subsequently becoming a highly pressing problem not only in dentistry and maxillofacial surgery but also in pharmacotherapy with these drugs in general (Spevak and Tsymbal 2017).

It is important to note that in foreign literature, the synonym "ischemic (avascular) bone necrosis" is used to designate ONJ, which emphasizes the vascular component of the etiology of the disease (Kos 2015). The following possible mechanisms for the development of ONJ are described in the literature: (1) impaired differentiation of stromal cells, leading to an increase in the number and size of adipocytes but not osteoblasts; (2) impaired vascular supply of intra- and extravasal origin; (3) deterioration in the "quality" of bone tissue itself due to generalized or local osteoporosis; (4) impaired intravascular coagulation factors that promote microthrombosis. A vascular (antiangiogenic) type of ONJ was also identified based on pathomorphological examination of surgical material from patients (Spevak and Tsymbal 2017). Based on this list, we note that we obtained data consistent with all of these pathogenetic mechanisms using zoledronic acid monotherapy with two doses. We identified pronounced lipomatosis of the bone marrow spaces with an increase in both the number and size of adipocytes. Bone trabeculae, despite the absence of significant changes in morphometric parameters, were characterized by clear signs of deterioration in bone quality and damage to osteocytes and the lacunar-tubular system. Vascular changes – angiosclerosis, intimal damage, reduction of intraosseous capillaries, and hemorheological disturbances including microthrombosis – are pronounced. Given that similar changes have also been observed in untreated osteoporosis, it can be assumed that the mechanisms of osteoporosis progression and damage caused by prolonged exposure to zoledronic acid are cumulative. Having provided a pronounced anti-osteoporotic effect in the early period with the first intervention in impaired bone remodeling, repeated pharmacological intervention in ongoing pathological processes in the late period leads to more severe side effects. Resveratrol therapy, in the context of changes caused by zoledronic acid side effects, demonstrated efficacy against all of the above-mentioned pathogenic components.

These components seemed to accentuate the pharmacological effects of resveratrol, the most important of which are:

1) Influence on the processes of proliferation, commitment, and differentiation of stromal bone marrow precursors, directing them along the osteoblastic pathway and inhibiting the adipocyte pathway. The result is a reduction in bone marrow adiposity and, therefore, likely a mitigation of the pathological effects of adipose tissue, both lipotoxic and inflammatory. The shift toward the osteoblastic lineage is manifested by structural and functional features of neoosteogenesis.

The significant role of the induction of adipogenesis in the osteoprotective effect of resveratrol is supported by modern concepts of metabolic pathology and skeletal aging. In one review publication from recent years, the title of which contains the intriguing term

"inflammaging" (combining "inflammation" and "aging") (Aaron et al. 2022), the following hypothesis is proposed based on the analysis of 130 published studies. With age, dysfunction of stromal stem cells develops, external factors of which include structural disintegration of bone marrow niches due to adipocyte expansion and increased secretion of proinflammatory cytokines (IL-1, TNF-α, RANKL, leptin) into the microenvironment, which leads to enhanced osteoclastogenesis. Increased PPAR-γ production leads to a switch of mesenchymal precursors toward the adipocyte differentiation pathway at the expense of osteoblastic differentiation. The authors characterize the resulting condition, with elevated concentrations of proinflammatory cytokines and adipokines in the bone marrow microenvironment, as low-grade chronic inflammation. Against this background, a combination of stimulated osteoclastogenesis and osteoblastogenesis failure develops, leading to bone loss. The accumulation of senescent cells also plays a significant role. According to the provisions of this hypothesis, therapeutic strategies include antioxidants, post-transcriptional interventions using microRNA, and the use of so-called senolytics, i.e., eliminators of senescent cells. This possibility was experimentally demonstrated by Baker et al. (2011) in progeroid Bub mice using the R1 INK-ATTAC transgene, which removes p16Ink4a-positive senescent cells in adipose tissue, skeletal muscle, and the eye, where they are involved in the development of age-associated pathologies. Lifelong removal of p16Ink4a-expressing cells delays the formation of senile phenotypes and even weakens the manifestations of already established pathological conditions.

There is a body of data indicating that the previous concept of adipose tissue as a protective factor against osteoporosis is no longer relevant, and a more modern concept considers bone marrow adiposity as a component of osteoporosis pathogenesis via lipotoxicity. Obesity should not be considered a protective factor against osteoporosis. Moreover, studying the relationship between bone and fat at the molecular and cellular levels will likely lead to a better understanding of various risk factors and the underlying pathogenesis, as well as to the development of drugs with dual mechanisms of action against osteoporosis and obesity in the future. Importantly, within pathogenesis frameworks, bone marrow adiposity is considered a variant of lipotoxic pathology, the development of which is driven by adipokines and proinflammatory cytokines. Lipotoxic organ damage, which includes "ectopic obesity" and a complex of biochemical disturbances associated with adipose tissue, is considered a significant, if not the primary, mechanism in the pathogenesis of metabolic diseases.

2) Cytoprotective action on osteocytes and their switching to a synthetic phenotype, which causes the absence of potential activation of osteoclasts by osteocyte death products and blockade of periosteocytic osteolysis. Positive effects on osteoblasts and osteocytes lead to the restoration and maintenance of the structural and biomechanical quality of bone substance.

3) Angioprotective action on bone vessels, most likely due to the endothelioprotective properties of resveratrol with the elimination of endothelial dysfunction and its characteristic vasomotor, hemorheological, and post-inflammatory disorders.

Conclusion

Single intravenous administration of zoledronic acid at a dose of 0.006 mg per 100 g of the animal's body weight to female Wistar rats with developing experimental postmenopausal osteoporosis inhibits the development of lesions in the femoral head, but prolonged therapy with two doses on the 4th and 8th weeks after ovariectomy leads to adverse effects, including damage to bone elements, intraosseous vessels, and bone marrow adiposity. Intraperitoneal resveratrol at an endothelial-protective dose of 2 mg/kg daily, starting after the second ZA injection for up to 12 weeks, exerts a pronounced therapeutic effect, affecting all bone, marrow, and vascular components of the bone.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interest.

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Ethics statement

The study was approved by the Animal Ethics Committee of Belgorod State National Research University (BelSU), Expert Opinion No. 02-01i/25 dated January 9, 2025.

Data availability

All data supporting the findings of this study are available within the main text.

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