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Effects of alendronate and risedronate therapy on hepatic antioxidant enzyme activity and lipid peroxidation in ovariectomized rats

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Aim: To evaluate the effects of alendronate (ALN) and risedronate (RIS), which are agents for preventing and treating postmenopausal osteoporosis, on hepatic antioxidant enzyme activity and lipid peroxidation in ovariectomized rats.

Materials and methods: Thirty-two female rats were randomly assigned to 4 separate groups. All of the groups, except the control group, were ovariectomized. Included in the 4 groups were a control group (C), an ovariectomized group (OVX), an ovariectomized group treated with alendronate (OVX-ALN), and an ovariectomized group treated with risedronate (OVX-RIS). Ten weeks after an ovariectomy, the OVX-ALN rats were administered 1.75 mg/kg of their body weight with alendronate sodium; and the OVX-RIS rats were administered 0.5 mg/kg of their body weight with risedronate sodium. Both doses were administered by gavage twice per week for 12 weeks. At the end of the treatment period, the liver enzyme activity levels of superoxide dismutase (SOD) and catalase (CAT) and malondialdehyde (MDA) levels were measured.

Results: There were significantly elevated levels of MDA observed in the OVX and OVX-ALN groups, whereas the MDA concentration significantly decreased in the OVX-RIS group when compared to the control animals. The SOD activity was significantly higher in the OVX and OVX-ALN groups, but there was no statistically significant difference in the SOD activity in the OVX-RIS group when compared with that of the control group. Moreover, CAT activity declined significantly in the OVX group and was elevated in the OVX-ALN group, according to the control group. No significant change in CAT activity was observed in the OVX-RIS group.

Conclusion: Alendronate increases the effect of ovariectomy on lipid peroxidation, and induces oxidative stress; however, risedronate prevents oxidative stress caused by an ovariectomy.

Key words: Alendronate, risedronate, ovariectomy, antioxidants

Overektomili sıçanlarda alendronat ve risedronat uygulamasının hepatik antioksidan enzim aktivitesi ve lipid peroksidasyonu üzerine etkileri

Amaç: Postmenopozal osteoporozu engellemek ve tedavi etmek amacıyla kullanılan alendronat (ALN) ve risedronat (RIS)'ın overektomili sıçanlarda hepatik antioksidan enzim aktivitesi ve lipid peroksidasyonu üzerine olan etkisini değerlendirmek.

Yöntem ve gereç: Otuz iki dişi sıçan kontrol grubu (C), overektomili grup (OVX), alendronat uygulanan overektomili grup (OVX-ALN) ve risedronat uygulanan overektomili grup (OVX-RIS) şeklinde randomize olarak ayrıldı. Kontrol grubu hariç tüm gruplara overektomi uygulandı. Overektomiden 10 hafta sonra, OVX-ALN grubuna 1,75 mg/kg alendronat sodyum and OVX-RIS grubuna ise 0,5 mg/kg risedronat sodyum gavaj yoluyla 12 hafta verildi. Uygulama sonunda karaciğer superoksit dismutaz (SOD), katalaz (CAT) enzim aktiviteleri ve malondialdehit (MDA) düzeyleri ölçüldü.

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Bulgular: OVX ve OVX-ALN gruplarında MDA düzeyleri kontrol grubu ile karşılaştırıldığında anlamlı derecede yükselmiş iken OVX-RIS grubunda MDA düzeyi anlamlı derecede düşmüş olarak gözlendi. SOD aktivitesi ise kontrol grubuyla karşılaştırıldığında OVX ve OVX-ALN gruplarında anlamlı derecede yükselmiş iken OVX-RIS grubunda anlamlı bir farklılık gözlenmedi. CAT aktivitesi kontrol grubuna göre OVX grubunda anlamlı derecede düşmüş iken OVX-ALN grubunda yüksek gözlendi. CAT aktivitesi OVX-RIS grubunda anlamlı bir farklılık göstermedi.

Sonuç: Alendronat overektominin lipid peroksidasyonu üzerine etkisini arttırır iken, risedronat overektominin neden olduğu oksidatif stresi engellemektedir.

Anahtar sözcükler: Alendronat, risedronat, overektomi, antoksidan

Introduction

Bisphosphonates are commonly prescribed to stabilize bone loss caused by osteoporosis in millions of postmenopausal women. The strategy in the treatment of osteoporosis is to inhibit the resorption of trabecular bone by osteoclasts, and hence preserve its density. For this purpose, oral bisphosphonates need to be prescribed and include etidronate, risedronate, tiludronate, and alendronate (1,2).

The bisphosphonates share the pyrophosphate structure, which characterizes the pharmacological group. They are structural analogs of pyrophosphates with specific activity upon bone. Bisphosphonates were formerly classified according to the chemical group added to the base pyrophosphoric nucleus on its R2 side chain (3-6). The second generation includes the aminobisphosphonates with a terminal amino group (e.g. alendronate and pamidronate), while the third generation is characterized by having a cyclic side chain, as in the case of risedronate (3).

Alendronate and risedronate are considered first-line therapy for the prevention and treatment of osteoporosis in postmenopausal women, as well as for the treatment of osteoporosis in men. Alendronate (4-amino-1-hydroxybutylidine-1,1-bisphosphonate) increases bone mineral density (BMD), and decreases fracture incidents over 4 years in postmenopausal women with osteoporosis (7-9). These beneficial clinical effects are associated with a marked antiresorptive effect, characterized by decreased bone remodeling by up to 90%. Alendronate and risedronate are also approved for the treatment of glucocorticoid induced osteoporosis in men. Risedronate is a pyridinyl bisphosphonate that exerts its clinical effects through binding to hydroxyapatite in bone tissue and inhibiting osteoclast activity (10,11).

Reactive oxygen species (ROS) may play important roles in various biological reactions, and they have been suggested to be effective in the pathogenesis of many diseases. ROS may cause tissue damage by affecting the cell membrane, genetic material, enzymatic pathways, and connective tissue structures. The relationship between ROS and human diseases is dependent upon the balance between the ROS and antioxidants. Cells are protected against oxidative damage by various systems (enzymatic, nonenzymatic), and molecules, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), are well-known scavenger enzymes that protect the cell from oxidative stress (12-14).

It has been suggested by several researchers that enzymatic and/or non-enzymatic antioxidant systems are impaired in osteoporosis, and thus osteoporotic patients are exposed to oxidative damage (15,16). The different activities of antioxidant enzymes, namely SOD and GSH-Px, have been reported with osteoporosis (16-19). It has also been supposed that osteoporotic patients are more prone to lipid peroxidation because of a reduced antioxidant defense system (17,19).

The objective of this study was to investigate whether alendronate and risedronate could influence the lipid peroxidation and antioxidative property in the livers of ovariectomized rats. To the best of our knowledge, the effects of alendronate and risedronate on lipid peroxidation, and antioxidant enzyme levels in ovariectomized rats have not yet been studied.

Materials and methods

All experimental protocols were approved by the Mersin University School of Medicine Animal Care and Use Committee. Sprague-Dawley female rats

(200-250 g) were kept in a room with a constant temperature of 22 ± 1 °C, and 12 h light and dark cycles, they were fed a standard rat chow, and had access to water ad libitum.

This study was performed on 4 groups of animals with each group consisting of 8 rats: (1) control group (C), (2) the ovariectomized group (OVX), (3) the ovariectomized group treated with alendronate (OVX-ALN), and (4) the ovariectomized group treated with risedronate (OVX-RIS).

Thirty-two rats were anesthetized with ketamine (Ketalar, Eczacıbaşı Pharmaceutical Co.) and then underwent a bilateral ovariectomy via ventral incision. Ten weeks after the ovariectomy, the OVX-ALN rats were administered 1.75 mg/kg of their body weight alendronate sodium, and the OVX-RIS rats were administered 0.5 mg/kg of their body weight risedronate sodium. Both doses were administered by gavage twice per week for 12 weeks. The BMD measurement is widely used for detecting osteoporosis. For this reason, we used dual-energy X-ray absorptiometry (Norland XR 45, Norland Scientific Instruments, Fort Atkinson, WI, USA) with a scan speed of 1 mm/s, and a resolution of 0.5 × 0.5 mm. Before taking the measurement, the instrument was calibrated by means of a Norland phantom. The BMD (in milligrams per square centimeter) was determined by the analysis of the femoral shaft.

After the treatment, the animals were killed by decapitation under ketamine anesthesia. The livers were quickly excised, rinsed in ice-cold 0.175 M KCl/25 mM Tris–HCl (pH 7.4) to remove the blood, weighed, finely minced in the same solution, and homogenized by means of a homogenizer with a Teflon pestle. The liver homogenates were centrifuged at 10,000 ×*g* for 15 min. The supernatants were then used for lipid peroxidation determination, and antioxidant enzyme assays.

Tissue SOD and CAT activity determination

The SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2^- generated by the xanthine/xanthine oxidase system (20). One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate.

The CAT activity of tissues was determined according to the method of Aebi (21). The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The enzyme activity is given in U/mg of protein.

Determination of malondialdehyde levels

The levels of malondialdehyde (MDA) in homogenized tissue, as an index of lipid peroxidation, were determined by a thiobarbituric acid reaction using the method of Yagi (22).

Determination of protein content

The tissue protein content was measured by a procedure similar to that documented by Lowry et al. (23) using bovine serum albumin as a standard.

Statistical analysis

The statistical analysis was carried out using SPSS 11.0. After obtaining normal distribution (Kolmogorov-Smirnov), the data were expressed as a mean ± standard deviation (SD), and the Bonferroni significant difference test was used to compare different groups. Values of $P < 0.05$ were regarded as significant.

Results

The BMD values were 0.145 ± 0.025 in the control group, 0.103 ± 0.044 in the OVX group, 0.141 ± 0.047 in the OVX-ALN group, and 0.146 ± 0.031 in the OVX-RIS group. The BMD value in the OVX group was significantly lower than that in the other groups ($P < 0.05$).

The results regarding lipid peroxidation and antioxidant activity are presented in the Table. The liver's MDA levels were significantly increased ($P < 0.05$) in the OVX and OVX-ALN groups, whereas the MDA concentration was significantly decreased ($P < 0.05$) in the OVX-RIS group compared to the control group. The SOD activity was found to be higher in the OVX and OVX-ALN groups; however, there was no statistically significant difference in the SOD activity in the OVX-RIS group when compared with that of the control group. Furthermore, CAT activity declined significantly in the OVX group and was elevated in the OVX-ALN group compared to the

Table. Liver MDA concentration, and SOD and CAT activity in the control, OVX, OVX-ALN, and OVX-RIS groups.

Variables	Control (n = 8)	OVX (n = 8)	OVX-ALN (n = 8)	OVX-RIS (n = 8)
MDA (nmol/mg protein)	1.50 ± 0.66	2.38 ± 0.39*	3.94 ± 1.74*,a	0.48 ± 0.12*,a
SOD (U/mg protein)	10.61 ± 1.62	19.22 ± 2.31*	42.32 ± 2.54*,a	13.72 ± 1.87
CAT (U/mg protein)	502.31 ± 56.13	276.43 ± 80.75*	783.38 ± 96.58*,a	496.23 ± 36.59a

Data are means ± SD
* Significant difference from control at $P < 0.05$
[a] Significant difference from OVX at $P < 0.05$

control group. No significant change in CAT activity was observed in the OVX-RIS group (Table). When the OVX group was compared to the OVX-ALN and OVX-RIS groups, the MDA concentration was significantly higher in the OVX-ALN treated animals, while it was lower in the OVX-RIS treated animals. The SOD activity increased in the OVX-ALN group; however, no significant variation in the SOD activity was obtained from the OVX-RIS group. The CAT activity was significantly elevated in both the OVX-ALN and OVX-RIS groups.

Discussion

Bisphosphonates are now well established as successful antiresorptive agents for the prevention and treatment of osteoporosis. In particular, alendronate and risedronate are approved as therapies for osteoporosis in many countries (7,24), and both drugs have a high affinity for bone mineral and function by reducing bone turnover by inhibiting osteoclast-mediated resorption (25-27). Clinical studies have demonstrated the clinical efficacy of alendronate and risedronate in reducing bone loss, and in the vertebral and non-vertebral fractures resulting from osteoporosis (28-31).

In addition to the effects of alendronate and risedronate on the bone in postmenopausal women, there are several studies that are related to the effects of alendronate, risedronate, and ovariectomy on the antioxidant system and lipid peroxidation separately (32-34). However, there are no reports concerning the influence of alendronate and risedronate on the antioxidant status and lipid peroxidation in ovariectomized rats. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids, and increased MDA content is an important indicator of lipid peroxidation (35).

In the present study, we used the ovariectomized rat model to show postmenopausal osteoporosis. The ovariectomized rat is the most frequently used model for osteoporosis (36,37). This model exhibits a progressive loss of bone matrix through a process that is similar to that which occurs during postmenopausal osteoporosis (36,38,39).

The results of the present study show that the liver's MDA levels and the SOD activity were significantly higher; however, CAT levels were significantly lower in the OVX group than in the control group. The increased SOD and reduced CAT in the OVX group would lead to the accumulation of H_2O_2 (40), a highly toxic metabolite for cells (41). H_2O_2 may be converted to H_2O, either by CAT or GSH-Px. H_2O_2 is decomposed to O_2 and H_2O by CAT at high concentrations, while GSH-Px serves for H_2O_2 degradation at lower concentrations (42). Observation of lower CAT levels in the OVX rats suggests that OVX may inhibit CAT activity and cause H_2O_2 accumulation, thus resulting in oxidative damage. Muthusami et al. (43) examined oxidative stress parameters in rat bone tissue homogenates after a bilateral ovariectomy. They demonstrated that SOD and GSH-Px activity decreased while lipid peroxidation as well as H_2O_2 concentration increased in the bones of ovariectomized animals when compared to the control group. Ha (44) showed that ovariectomy increased the levels of MDA and decreased levels of the antioxidative enzymes SOD, CAT, and GSH-Px. In our previous study (34), we found that OVX leads to an increase in MDA levels and a decrease in the SOD and CAT activity in the livers of ovariectomized rats. A decrease in the activity

of any one of the antioxidant enzymes would likely lead to cellular death due to an accumulation of H_2O_2, which is an extremely cytotoxic chemical species. An ovariectomy leading to estrogen insufficiency results in general changes in metabolism that can be seen in the liver. The lack of protective estrogen is reflected in the alterations of an antioxidative/oxidative balance in the liver (45).

In the OVX-ALN group enhanced MDA levels as well as SOD and CAT activity in the osteoporotic livers of all groups were observed. The increase in MDA concentration, in spite of increased SOD and CAT activity, could have been due to the overproduction of ROS that exceeded the capacity of these antioxidant enzymes. In the literature, there are limited studies related to the influence of alendronate on lipid peroxidation and antioxidants. Şener and coworkers (32) showed that MDA concentration increased in the alendronate group, and they reported that alendronate induced oxidative gastric damage in rats by increasing lipid peroxidation and decreasing glutathione levels. Our results are in agreement with the data of Sener et al. (32).

In the OVX-RIS group, the MDA levels decreased and the activity of SOD and CAT remained unchanged when compared to the control group. The unchanged antioxidant enzyme activity may be due to the declined MDA levels, and, as a result, the SOD and CAT enzymes may not be induced. These findings suggest that risedronate can prevent oxidative damage by decreasing the MDA concentration. Ozgocmen et al. (33) assessed the in vivo effects of calcitonin, risedronate, and raloxifene on the erythrocyte oxidant-antioxidant status in women with postmenopausal osteoporosis. They showed that MDA levels declined but the activity of SOD and CAT were not changed by the administration of risedronate (33).

Alendronate is used in many countries to treat osteoporosis, and it has been extensively evaluated in clinical trials, lasting up to 10 years (7,8). Our results demonstrated that alendronate increases the effect of an ovariectomy on lipid peroxidation and induces oxidative stress in the liver of ovariectomized rats. However, when risedronate was given to ovariectomized rats, the lipid peroxidation decreased when compared to the alendronate treated group, thus suggesting a decrease in oxidative stress. As a result, it may be suggested that risedronate possesses some antioxidant properties, and can contribute to the effectiveness of therapy for osteoporosis.

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