Effect of melatonin on some blood and bone minerals contents in aged female rats

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Abstract

Aging influences bone resorption and formation. There is a strong correlation between decreased melatonin (MEL) levels and the increased incidence of bone deterioration. The aim of the present work was to examine MEL on blood and bone minerals contents. Twenty female rats (24 months old) were divided into two equal groups, vehicle-treated control and MEL treated (subcutaneous injection of 50 µg/100 g b.w MEL). After 14 weeks of treatment, blood and bone samples were collected. Serum total and bone specific alkaline phosphatase (TALP and BALP), corticosterone, nitrite (NO), malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) activity and serum and bone calcium (Ca), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) were determined. There was a significant (P<0.01) increase in bone mass, BALP and SOD activities and GSH in MEL treated rats. Moreover, a significant (P<0.05) increase in serum Ca level and serum and bone Zn and Fe and significant (P<0.05) decrease in serum NO, MDA and corticosterone level in treated group was observed. In conclusion, long-term melatonin treatment has a positive impact in preventing aging related bone degradation and lipid peroxidation.

Keywords: Melatonin; bone; aging; minerals, lipid peroxidation


Introduction

Essential trace minerals such as copper (Cu), manganese (Mn) and zinc (Zn) are important for the maintenance of healthy normal bone tissue. They are required in the formation of the bone framework structure contributing to the organic component of the osseous matrix. The normal bone hard mass is formed by inorganic minerals such as calcium (Ca) and phosphorous (P), hydroxyapatite, a structure referred to as the mineral mass (Ovesen et al., 2004), which is deposited around a structural framework named the “organic bone matrix” (Brodsky and Persikov, 2005). This matrix is composed of proteins which require iron (Fe), Zn, Mn and Cu as essential co-factors for enzymes involved in their synthesis (Tuderman et al., 1977; Ovesen et al., 2004).

Melatonin (MEL) hormone is rhythmically produced in the pineal gland controlled by the suprachiasmatic nucleus (SCN) and the light/dark cycle (Zawilska et al., 2009). MEL has a regulatory role in many physiological processes including bone physiology (Gitto et al., 2009). These effects had been studied relative to bone remodeling (Cardinali et al., 2003) and osteoporosis (Kotlarczyk et al., 2012). In addition to the sex steroid decrease that occurs with age and the changes in the levels of many well-studied local and systemic factors in perimenopausal women, decline in MEL secretion could also play a role in the aetiology of osteoporosis (Ostrowska et al., 2002). Moreover, lighting conditions, pinealectomy and long-term administration of MEL caused a modification of circadian bone metabolism in rats (Ostrowska et al., 2002).

It had also been shown that increased formation of the hormone during the day, as observed in obesity, may positively affect the bone after the menopause (Ostrowaska et al., 2008). In addition to some in vivo
and in vitro animal and human studies, which suggest that MEL may directly and/or indirectly affect both the formation and resorption of bone (Ostrowska et al., 2008).

A strong correlation between declined plasma MEL levels and the increased incidence of bone deterioration as seen in post-menopausal women has been reported (Ostrowska et al., 2001). Moreover, Ostrowska et al. (2002) reported that pinealectomy in rats affects bone metabolism biomarkers. Furthermore, Feskanich et al. (2009) showed that twenty or more years of nightshift work caused a marked increase in the risk of wrist and hip fractures in post-menopausal women. Clinical studies have focused on the possible therapeutic value of melatonin in the prevention or treatment of osteoporosis most experiments were performed in ovariectomized rats as a model for menopause (Uslu et al., 2007). The aim of the work was to study the effect of MEL treatment in aged female rats on bone mineral contents (BMC) and some markers of blood and bone metabolism.

Materials and Methods

The diagnostic kits assaying SOD activity, levels of GSH, MDA and NO were obtained from Bio-diagnostic Company, Egypt. Kit assaying levels of total and bone, ALP were purchased from Diamond Diagnostic, Egypt.

Experimental animals

This study was conducted on twenty aged (24 months) female albino rats weighing 300-345 g. Rats were randomly assigned into two groups (10 rats/group) and housed in wired cages. Water and food (standard laboratory diet containing 0.5% NaCl, 22% protein and 4-6% dietary fat) was obtained from Damanhour Feed Co, Behera, Egypt. Rats received humane care in compliance with the guidelines of animal care of the National Institutes of Health (NIH), Alexandria University. The local committee of the Faculty of Veterinary Medicine, Alexandria University approved this study. Rats were kept at a natural humidity, light cycle and room temperature 22-25°C.

Experimental design

Rats in the first group received MEL subcutaneous injection of 50 µg/100 g b.w (MEL, M-5250 Sigma, USA) (Ostrowska et al., 2002) which was dissolved in 0.5 ml of 5% solution of ethyl alcohol in physiological saline. Rats in the second group (control) received 0.5 ml of 5% solution of ethyl alcohol in physiological saline. For both groups, the treatment was conducted on alternative day for 14 weeks.

Blood and bone tissue sampling

Twenty four hours after the last injection, rats were killed with light ether anesthesia and 12 h fasting, blood samples were obtained for separation of serum which was stored at -20°C for determination of total and bone specific alkaline phosphatase, corticosterone and trace elements. Tibiae and femurs were dissected, placed in plastic bags, and stored frozen until analysis.

Bone mass measurement

Alternate left and right tibiae and femurs were immersed in a mixture of chloroform and methanol solvent (2:1 by volume, respectively) for one week to remove the fat from the bones (Umemura et al., 2008). The bones were oven dried at 80°C for 24 h and then weighed using an electronic balance (Setra Systems, INC. USA-BL-410S).

Determination of trace elements in serum and bone

Serum samples were digested by concentrated acids (3 ml perchloric acid + 2 ml nitric acid per ml) for 24 h. Bones were ashed in a furnace at 550°C. Then the residues were digested by concentrated acids (Fayed, 2010). All samples were filtered and analyzed for the concentration of Ca, Zn, Cu, Fe, and Mn by flame emission atomic absorption spectrophotometer (Model 210 VGP Buck Scientific, USA).

Total ALP activity assay

Total ALP activity was measured by a kinetic method according to the manufacturer instruction. P-nitrophenyl phosphate (PNPP) was used as substrate. Activity was measured as dA/min at 504 nm.

Bone specific ALP

The heat inactivation method shows lower stability of the bone specific ALP isoform at 56°C over 15 min. 200 µl of serum (with known total ALP activity) in plastic Epindorf tubes were placed in a water bath at 56°C for 15 min. The samples were then cooled on ice to 22°C and duplicate 25 µl aliquots were removed for the measurement of heat-insensitive ALP isoenzyme. Heat-insensitive ALP activity was determined by the same method described for total ALP. Inactivated ALP (BALP) was calculated by subtracting the activity of the heated sample from the total activity of the unheated sample (Farley et al., 1993).

Estimation of serum MDA and GSH

Level of lipid peroxidation product (MDA) was measured based on the formation of thiobarbituric acid-reactive substances (TBARS) using the method of Ohkawa et al. (1979). Level of reduced glutathione was measured based on the reduction of 5, 5” dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione according to the method of Glatzle et al. (1974).

Estimation of serum SOD

Estimation of SOD activity in serum relied on the ability of the enzyme to inhibit the phenazine
methosulfate-mediated reduction of nitro blue tetrazolium dye and measured the increase in the absorbance at 560 nm for 5 min for the control (ΔAcontrol) and the sample (ΔAsample) at 25°C according to the method of Nishikimi et al. (1972).

Assessment of serum NO concentration

NO in serum was colorimetrically estimated according to the method described by Montgomery and Dymock (1961). The nitrite concentration is an indicator of NO production. The method used is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase, followed by Griess reaction which converts nitrite into a deep purple azo compound that was photometrically measured at 540 nm.

Estimation of serum corticosterone

Serum corticosterone was determined according to the method of Krame and Sothern (2001) using commercial enzyme immunoassay kit (IBL International GMBH Flughafenstassa, Hamburg, Germany).

Statistical analysis

Results were expressed as mean±SE. All data were analyzed using independent t-test by the aid of SAS (2002) software for control and treated groups.

Results

Effect of melatonin treatment on serum corticosterone, TALP, BALP, MDA, GSH, NO and SOD

In the present study, data presented in Table 1 showed that MEL treatment in aged female rats caused a significant (P<0.05) decrease in corticosterone level, activity of TALP, levels of MDA and NO compared to control. MEL also caused a significant increase (P<0.05) in the activity of BALP, and a significant increase (P<0.01) in SOD activity and level of GSH compared to control group.

Effect of MEL on serum Ca, Cu, Fe, Zn and Mn

Aged female rats treated with MEL exhibited a significant (P<0.05) increase in serum Ca, Zn and Fe compared to control. Serum Cu and Mn showed non-significant (P>0.05) changes between MEL and control group (Table 2).

Effect of MEL treatment on bone mass, and some bone mineral contents (Ca, Zn, Fe, Cu and Mn) in tibia and femur

In both tibiae and femurs, MEL treatment resulted in a significant (P<0.01) increase in bone mass (g) compared to control (Table 3). Also data presented in Table 3 showed a significant (P<0.05) increase in Zn and Fe contents in both tibiae and femurs of MEL treated rats compared to control. On the other hand Ca, Cu and Mn levels in MEL treated rats showed no significant change compared to control (Table 3).

Discussion

In the present study treatment of aged female rats with MEL caused a significant increase in the weight of tibia and femur bones compared to their corresponding weights in control rats. Bone mass accounts for 50-70% of bone strength (Pocock et al., 1987). The decrease in tibiae and femurs weight in the aged control rats agree with the findings of Wang et al. (2011). They reported that the reduction of bone mass and consequently bone strength in aged rats was probably due to the aging increase in bone resorption. The increase in tibiae and femurs weight after MEL treatment agrees with the previous findings of Adizesky et al. (2006) and with the previous findings of Suzuki et al. (2008) who reported that MEL treatment caused an increase in trabecular thickness and trabecular area of vertebra and femur and cortical thickness of femur, in ovariectomized rats animal model, which were significantly reduced after ovariectomy (Uslu et al., 2007). The increase in tibial and femoral weight after MEL treatment could be related to MEL stimulation of skeletal growth and bone formation, most probably through inhibition of bone resorption by down-regulation of RANKL-mediated osteoclast activation (Histing et al., 2012) or augmentation of the total bone mineral density and
compared to control values. Also bone Ca contents in levels showed non significant changes in MEL group treated group.

increase in MEL treated compared to control aged both tibia and femur exhibited a non significant significant increase in bone Zn and Fe contents in MEL treated rats which might be the result from a different mechanism than increase in osteoclast activation (Hist ing et al., 2012). The present findings suggest, that the positive effects of MEL on bone metabolism could be related not only to its direct effect on bone cells but also to its powerful antioxidative effect (Reiter et al., 2009) and its ability to inhibit inducible nitric oxide synthase (iNOS). iNOS plays a critical role in the pathogenesis of osteoporosis since it promotes the generation of NO, a free radical which contributes to bone resorption caused by oestrogen depletion. MEL also directly neutralizes free radicals and stimulates the activity of antioxidative enzymes and protects bone cells from oxidative attacks. Osteoclasts generate high levels of superoxide anions during bone resorption that contribute to the degradation process (Gomez-Moreno et al., 2010).

In the present work serum levels of corticosterone showed a significant (P<0.05) decrease in MEL group compared to control levels which might be one of

contents (Adizesky et al., 2006) which is consistent with the findings of the present study as there was a significant increase in bone Zn and Fe contents in MEL treated group.

In the present study, serum and bone Cu and Mn levels showed non significant changes in MEL group compared to control values. Also bone Ca contents in both tibia and femur exhibited a non significant increase in MEL treated compared to control aged female rats. On the other hand, serum Ca levels showed a significant increase in MEL group compared to control values. The increase in serum Ca following MEL treatment is similar to the findings of Ostrowska et al. (2003) and Adizesky et al. (2006) which indicate an up regulation of MEL to blood Ca level (Ostrowska et al., 2003). Probably based on the presence of existed evidence for the pineal control of the secretion of parathyroid hormone and calcitonin, confirmed by the ultrastructural and functional changes observed in parathyroid glands after pinealectomy (Hakanson and Bergstrom, 1981). On the other hand, the recorded insignificant changes in bone Ca contents in MEL treated female rats compared to control could be due to inhibition of bone resorption by down-regulation of RANKL-mediated osteoclast activation (Hist ing et al., 2012), suggesting that the increase in serum Ca level might be the result from a different mechanism than increase bone resorption.

The significant increase in serum and bone Zn and Fe level after MEL treatment reflects the positive impact of MEL on bone physiology and formation of mineralized matrix in bone (Adizesky et al., 2006; Sethi et al., 2010). Iron is a cofactor for prolyl and lysyl hydroxylases, enzymes that catalyze an ascorbate-dependent hydroxylation of prolyl and lysyl residues, essential steps for cross linking by lysyl oxidase (Tuderman et al., 1977). Moreover, rats fed an Fe deficient diet even mild deficiency showed a decreased mechanical strength in femurs and significant changes in cortical area and bone physiology, which may be attributed to impaired function of prolyl and lysyl hydroxylase (Medeiros et al., 2002) leading to a significant impact upon bone mineralization, decreasing the matrix formation and increasing bone resorption (Diaz-Castro et al., 2012).

Zn plays an important role in every step of bone metabolism, acting as a cofactor, stimulating protein synthesis and required for organic matrix formation mainly collagen (Doherty et al., 2002), that form the structural framework around which mineralization occurs. The main mineral component of bone is a crystalline salt of calcium hydroxyapatite [Ca10 (PO4)6(OH)2], which also contains Zn in addition to other transition metals (Ovesen et al., 2004). Zn also functions as a metal component of ALP, a metalloenzyme that plays a key role in the formation of new bone. Zn may induce an increase in ALP-related DNA synthesis and, as a result, stimulates bone growth (Imamoglu et al., 2005). Moreover, in another study, Zn caused an increase in osteogenic effect by stimulating cell proliferation, ALP activity and collagen synthesis in osteoblastic MC3T3-E1 cells (Seo et al., 2010). The previous findings are consistent with the findings in the present study as there was a significant increase in BALP activity accompanying the significant increase in serum and bone Zn levels in MEL treated rats which gives a reasonable explanation for the stimulatory effect of MEL on osteoblastic activities, through increasing bone Zn contents and consequently BALP (Satomura et al., 2007; Mahjoub and Masrour, 2012).

There was a significant decrease in serum NO and MDA, in addition to increased GSH content and activity of SOD after MEL treatment. The present findings suggest, that the positive effects of MEL on bone metabolism could be related not only to its direct effect on bone cells but also to its powerful antioxidative effect (Reiter et al., 2009) and its ability to inhibit inducible nitric oxide synthase (iNOS). iNOS plays a critical role in the pathogenesis of osteoporosis since it promotes the generation of NO, a free radical which contributes to bone resorption caused by oestrogen depletion. MEL also directly neutralizes free radicals and stimulates the activity of antioxidative enzymes and protects bone cells from oxidative attacks. Osteoclasts generate high levels of superoxide anions during bone resorption that contribute to the degradation process (Gomez-Moreno et al., 2010).

In the present work serum levels of corticosterone showed a significant (P<0.05) decrease in MEL group compared to control levels which might be one of

Table 3: Effect of melatonin treatment on bone mass (tibia and femur weights) and Ca, Zn, Fe, Cu and Mn in tibia and femur

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Tibia weight (g)</th>
<th>Ca (mg/g)</th>
<th>Zn (µg/g)</th>
<th>Fe (µg/g)</th>
<th>Cu (µg/g)</th>
<th>Mn (µg/g)</th>
<th>Femur weight (g)</th>
<th>Ca (mg/g)</th>
<th>Zn (µg/g)</th>
<th>Fe (µg/g)</th>
<th>Cu (µg/g)</th>
<th>Mn (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.43±</td>
<td>78.61±</td>
<td>128.53±</td>
<td>71.69±</td>
<td>5.80±</td>
<td>0.29±</td>
<td>0.49±</td>
<td>75.51±</td>
<td>127.35±</td>
<td>69.74±</td>
<td>5.86±</td>
<td>0.30±</td>
<td></td>
</tr>
<tr>
<td>Melatonin</td>
<td>0.58±</td>
<td>85.19±</td>
<td>147.2±</td>
<td>83.88±</td>
<td>6.21±</td>
<td>0.31±</td>
<td>0.64±</td>
<td>86.92±</td>
<td>156.6±</td>
<td>82.05±</td>
<td>6.69±</td>
<td>0.26±</td>
<td></td>
</tr>
</tbody>
</table>

*All values are means ±SE, n=10; Values with *within the same column are significantly different (P<0.05); Values with ** within the same column are significantly different (P<0.01)
causes of decreased bone degradation. The influence of aging on rodent plasma corticosterone levels under basal conditions and in response to treatment with various stressors or ACTH have been subjected to extensive studies and the findings of these studies showed great controversy. It is apparent that both basal and stress-induced corticosterone levels differ markedly among various strains of rats and mice and also among the same strain. Some of these variations may be due to various compensatory mechanisms operating in rodent models, including increased or decreased disposal rates of the corticosterone or changes in binding proteins (Lo et al., 2000). The decrease in serum levels of corticosterone after MEL treatment agrees with the previous findings of Richter et al. (2007). Their findings indicated not only expression but also high amplitude diurnal variation of functional MEL receptors (MT1) in the rat adrenal gland in vivo and in vitro studies. They suggested that melatonin directly inhibits ACTH-stimulated corticosterone production in a clock time-dependent manner and the increase in plasma MEL would encounter functional adrenal receptors and could contribute to the down slope of the corticosterone rhythm. It is understood that glucocorticoids affect the function of all 3 cell types, osteoblasts, osteoclasts and osteocytes with most of the evidence indicating that osteoblasts are the main skeletal target (Henneicke et al., 2011). Glucocorticoids also suppress osteoblast function, including osteocalcin synthesis (Brennan-Speranza et al., 2012), which correlates with bone loss over time (Prummel et al., 1991) so that the decrease in corticosterone level in the present study could be one of the factors that minimize bone degredation.

In conclusion, the findings of the present study support a positive effect of MEL on bone formation and antioxidant status in aged rats.

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