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Anti-osteoporotic effects of melatonin and misoprostol in glucocorticoid-induced osteoporosis: An experimental study

Glukokortikoid kaynaklı osteoporozda melatonin ve misoprostolün anti-osteoporotik etkileri: Deneysel çalışma

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Abstract

Aim: Although there are some treatment options for glucocorticoid induced osteoporosis (GIO), new drug alternatives are still needed. In this study, we aimed to determine the protective effects of misoprostol and melatonin in an experimental GIO model.

Methods: The rats were grouped into four, with 10 rats in each group. The 1st group was chosen as the control group, which were not intervened with. Group 2 was the steroid group, group 3 the misoprostol group and group 4, the melatonin group. To the rats in groups 2, 3 and 4, 10 mg/kg subcutaneous methylprednisolone was administered for 28 days. To the rats of the 3rd group, 200 mg/day misoprostol was given per day by a cannula to the stomach. The rats in the 4th group received 5mg/kg intraperitoneal melatonin during this 28-days period. Lumbar vertebrae and femur bone mineral density (BMD) of all rats were measured by Dual-energy X-ray absorptiometry (DEXA) and assessed in pre- and post-treatment periods.

Results: In the steroid group, when the pre- and post-treatment-BMD values of the rats were compared, statistically significant decreases were found in vertebrae, whole femur, femur proximal, femur diaphysis and distal femur bone regions (P=0.011, P=0.005, P=0.007, P=0.005 and P=0.013; respectively). In the misoprostol group, a statistically significant decrease was observed only in the whole femur region (P=0.012) when the pre- and post-treatment BMD values of the rats were compared, while no significant changes were observed in vertebrae, femur proximal, femur diaphysis and distal femur bone regions (P=0.093, P=0.401, P=0.161 and P=0.123; respectively). In the melatonin group, when the pre- and post-treatment BMD values of the rats were compared, a statistically significant decrease was observed only in the vertebrae region (P=0.009), no significant changes were observed in whole femur, femur proximal, femur diaphysis and distal femur bone regions (P=0.386, P=0.445, P=1.000 and P=0.483; respectively).

Conclusion: Positive effects of misoprostol and melatonin on bone metabolism were determined in this experimental study. Misoprostol and melatonin seem to be potential agents that can be used in the prevention of GIO.

Keywords: Glucocorticoids, Osteoporosis, Melatonin, Misoprostol

Öz

Amaç: Glukokortikoid kaynaklı osteoporoz için kullanılan bazı tedavi seçenekleri olsa da, yeni ilaç alternatiflerine hâlâ ihtiyaç duyulmaktadır. Çalışmamızda ratlarda glukokortikoid kaynaklı osteoporoz modelinde misoprostol ve melatoninin koruyucu etkilerini belirlemeyi amaçladık.

Yöntemler: Ratlar her grupta 10'ar adet olacak şekilde dört gruba ayrıldı: Grup1 kontrol, grup 2 steroid, grup 3 misoprostol, grup 4 melatonin grubu olarak belirlendi. Grup 2, 3, 4'teki ratlara 28 gün boyunca 10 mg/kg/gün subkutan metilprednizolon uygulandı. Grup 3'teki ratlara ilave olarak 200 mg/gün orogastrik misoprostol, grup 4'teki ratlara ilave olarak 5 mg/kg/intraperitoneal melatonin uygulandı. Tüm ratların lomber vertebra ve femur kemik mineral yoğunluğu DEXA yöntemi ile ölçülerek tedavi öncesi ve sonrası değerler karşılaştırıldı.

Bulgular: Steroid grubunda, çalışma sonrası vertebra, tüm femur, femur proximal, femur diaphysis ve distal femur kemik mineral yoğunluğu değerleri istatistiksel olarak anlamlı derecede düşük bulundu (sırasıyla; P=0,011, P=0,005, P=0,007, P=0,005 ve P=0,013). Misoprostol grubunda sadece tüm femur bölgesinde çalışma sonrası kemik mineral yoğunluğu düşük (P=0,012) olup vertebra, femur proximal, femur diaphysis ve distal femur bölgeleri osteoporozdan korunmuştu (sırasıyla; P=0,093, P=0,401, P=0,161 ve P=0,123). Melatonin grubunda sadece vertebra bölgesinde çalışma sonrası kemik mineral yoğunluğu düşük (P=0,009) olup tüm femur, femur proximal, femur diaphysis ve distal femur bölgelerinde osteoporozdan korunmuştu (sırasıyla; P=0,386, P=0,445, P=1,000 ve P=0,483).

Sonuç: Bu deneysel çalışmada misoprostol ve melatoninin kemik metabolizması üzerindeki olumlu etkileri belirlenmiştir. Misoprostol ve melatonin GIO'nun önlenmesinde kullanılabilecek potansiyel ajanlar gibi görünmektedir.

Anahtar kelimeler: Glukokortikoid, Osteoporoz, Melatonin, Misoprostol

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Introduction

Glucocorticoids are commonly prescribed treatment modalities in chronic inflammatory and autoimmune diseases in all age groups. Unfortunately, one of the main side effects associated with the long-term glucocorticoid treatment is alterations in bone mineral density (BMD) causing an increase in fracture risk [1]. Glucocorticoid treatment has been shown to cause a decrease in bone vasculature and induce apoptosis in both osteoblasts and osteocytes resulting in structural deterioration of bone tissue and osteoporosis [2,3].

Glucocorticoid induced osteoporosis (GIO) has two main mechanisms: Excessive bone resorption with osteoclasts and impaired bone formation due to a decrease in osteoblast count and action [4]. Although some medications such as bisphosphonates or anabolic agents have been suggested together with the maintenance of adequate calcium intake and normal vitamin D status in prevention and/or treatment of GIO, a general consensus is yet to be reached. In especially children, it is clear that the treatment of osteoporosis is more difficult than prevention [5].

Misoprostol (15–deoxi-16-hydroxy-methyl PGE1) is a methyl derivative of prostaglandin E1. In experimental studies, misoprostol treatment was associated with an increase in bone turnover, with a net anabolic effect [6]. Osteo-inductive effects of misoprostol in the early bone healing period were defined [7]. Misoprostol has also been determined as an alternative treatment for osteopenia and osteoporosis in women during the postmenopausal period [8].

Melatonin, N-acetyl-5-methoxytryptamine, is a tryptophan derived indolamine hormone that is released by the pineal gland and found in many tissues. The anabolic effects of melatonin on bone remodeling by increasing osteoblast proliferation and differentiation and suppressing osteoclastogenesis was priorly determined [9,10]. Bone remodeling, the equilibrium between formation and resorption of bone tissue, mainly depends on the work of osteoblasts and osteoclasts in harmony. Oxidative stress has been shown to interfere with multiple cellular events that induce mesenchymal stem cell differentiation, promote apoptosis of mature osteoblasts and accelerate osteoporosis by increasing bone resorption and decreasing bone formation [11]. Melatonin with its potent anti-oxidant properties and cyto-protective effects may also be considered a protective hormone for oxidative-stress-induced mechanisms in osteoporosis [12].

Thus, in this experimental study, we aimed to determine the effects of misoprostol and melatonin individually, in prevention of GIO when concurrently applied with glucocorticoids. To the best of our knowledge, this is the first study in literature, evaluating the protective effects of misoprostol and melatonin in GIO.

Materials and methods

Animal samples

Two months old 40 Wistar female rats with a mean body mass of 143.8(20.1) grams were included in this randomized, controlled and double-blinded experimental study. Rats were fed with standard laboratory food and water for 28 days. The room in which the rats were cared for was 24(3)^{o}C with light and dark cycles of 12 h; rats were scaled every 10 days. Two rats from the 3rd group were lost due to cannula damage.

Ethical approval was obtained from Inonu University Medical Faculty ethics committee (2003/23). Then the rats were divided into 4 groups with 10 rats per each:

1st group was determined as control group, without application of any drugs;

2nd group was determined as the GIO (steroid) group, with the administration of 10 mg/kg subcutaneous methylprednisolone as a single dosage per day for 28 days;

3rd group was determined as the misoprostol group, with the administration of 200 μg/day prostoglandine E1 (misoprostol) directly to the stomach with a metal cannula together with 10 mg/kg subcutaneous methylprednisolone administration as a single dosage per day for 28 days;

4th group was determined as the melatonin group, with the administration of 5 mg/kg intraperitoneal melatonin along with 10 mg/kg subcutaneous methylprednisolone administration as a single dosage per day for 28 days.

At the beginning and at the end of the study, all rats were anesthetized with 8 mg Xylazinhydroclorur and 75 mg/kg ketamine for bone mineral density measurements. After 28 days, under halothane anesthesia, euthanasia was performed by drawing intracardiac blood.

All animal procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH).

Bone mineral density measurement

The bone mineral density (BMD) measurements of lumbar vertebrae and femur of all rats were performed using DEXA (QDR 4500/W, Hologic Inc., Bedford, MA, USA) and data was analyzed by the same researcher.

Our DEXA machine uses 70 kV and 140 kV double energized X-ray. For the analysis of all interest sites “subregion analysis software” was used. BMD was measured as g/cm^2 from four sites of the right femur and lumbar vertebrae. The lumbar vertebrae region (R1), total femur region (R2), adjacent 3 femur interest sites (R3=1/3 proximal, R4=1/3 mid-site, R5=1/3 distal) were charted. Superior iliac margin levels on lumbar areas were drawn in rectangles of 17x5 mm height and width. Femur interest sites were drawn with 3 mm width (Figure 1).

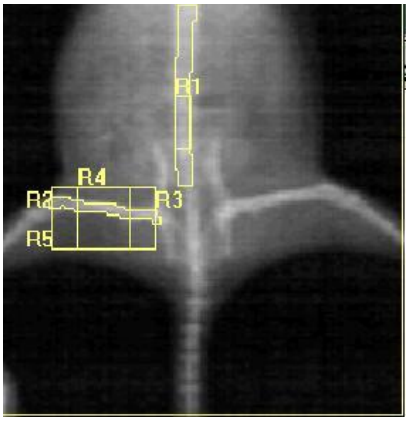

Figure 1: Interest sites specified and measured by DEXA

BMD rates showing BMD changes were calculated by using BMD data before and after the study. While a positive increase in BMD rates shows that post-study-BMD decreased, a negative increase in BMD rates shows that post study BMD increased.

$$\text{BMD rate} = \frac{\text{pre BMD} - \text{post BMD}}{\text{pre BMD}}$$

Statistical analysis

Statistical analysis was performed by using the statistical package for the social sciences version 17.0 (SPSS Inc., Chicago, IL, USA). Non-parametric tests were used for statistical analysis. Variables were shown as median and minimum-maximum values. Mann Whitney U test was used for comparisons between two independent groups and Kruskal-Wallis test was used for comparison of independent 3 or more groups. The post-hoc multiple comparisons with Mann-Whitney U test were statistically significant at $P<0.016$ for 4 groups. The significance of the differences between the two dependent samples was evaluated by Wilcoxon signed ranks test. $P<0.05$ was considered statistically significant.

Results

Groups were analyzed for BMD with DEXA measurements from R1, R2, R3, R4 and R5 interest sites and assessed as before treatment and after treatment. Data from all groups were assessed individually. Data before and after treatment in all groups are summarized in Table 1.

There were no statistically significant differences between the weight measurements of the rats before and after the study in control, steroid, misoprostol and melatonin groups (P=0.443, P=0.066, P=0.400 and P=0.445; respectively).

The pre-and post-study comparison of vertebrae, whole femur, proximal femur, femur diaphysis and distal femur BMD values of the rats in the control group revealed no changes, as expected (P=0.169, P=0.646, P=0.906, P=0.959 and P=0.241; respectively). In the steroid group, statistically significant decreases were found between pre- and post-treatment vertebrae, whole femur, proximal femur, femur diaphysis and distal femur bone mineral densities (P=0.011, P=0.005, P=0.007, P=0.005 and P=0.013; respectively). In the misoprostol group, upon comparison of pre- and post-treatment BMD values, a statistically significant decrease was observed only in the whole femur region (P=0.012) and no significant changes were observed in vertebrae, proximal femur, femur diaphysis and distal femur bone regions (P=0.093, P=0.401, P=0.161 and P=0.123; respectively). In the melatonin group, when the pre- and post-treatment BMD values of the rats were compared, a statistically significant decrease was observed only in the vertebrae region (P=0.009) and no significant changes were observed in whole femur, femur proximal, femur diaphysis and distal femur bone regions (P=0.386, P=0.445, P=1.000 and P=0.483; respectively). Pre- and post-treatment alterations of BMD values in vertebrae and femur regions are shown in Figure 2.

BMD rates and intergroup comparison of the study groups in related bone areas with BMD measurements are shown in Table 2.

When the BMD rates were compared between the groups, there were statistically significant differences in whole femur region, proximal femur and femur diaphysis regions (P=0.005, P=0.048 and P=0.005; respectively). There were no statistically significant differences in BMD values of the vertebrae and distal femur regions between the groups (P=0.104 and P=0.126; respectively).

Post-hoc multiple comparisons of BMD values for whole femur, proximal femur and femur diaphysis are summarized in Table 3.

In post hoc multiple comparisons in Table 3, BMD values of whole femur bone region were compared between the groups and the BMD value of the steroid group was found significantly higher than the control and melatonin groups (P=0.007 and P=0.002; respectively). There were no statistically significant differences between the control, misoprostol and melatonin groups (P=0.051 and P=0.880; respectively), between the steroid and misoprostol groups (P=0.131) and between the misoprostol and melatonin groups (P=0.110). The BMD values of the proximal femur region of the steroid group were found significantly higher than the control and melatonin groups (P=0.010 and P=0.013; respectively). There were no statistically significant differences between the control, misoprostol and melatonin groups (P=0.248 and P=0.821; respectively), between the steroid and misoprostol groups (P=0.594) or between the misoprostol and melatonin groups (P=0.374) in the multiple comparison of the BMD values of the proximal bone region. The femur diaphysis BMD values of the steroid group were found significantly higher compared to the control group and melatonin group (P=0.003 and P=0.002; respectively). There were no statistically significant differences between the control, misoprostol and melatonin groups (P=0.328 and P=0.940; respectively), between the steroid and misoprostol groups (P=0.026) and between the misoprostol and melatonin groups (P=0.286). BMD values of groups in femur regions that is showing significant differences in intergroup comparisons are shown in Figure 3.

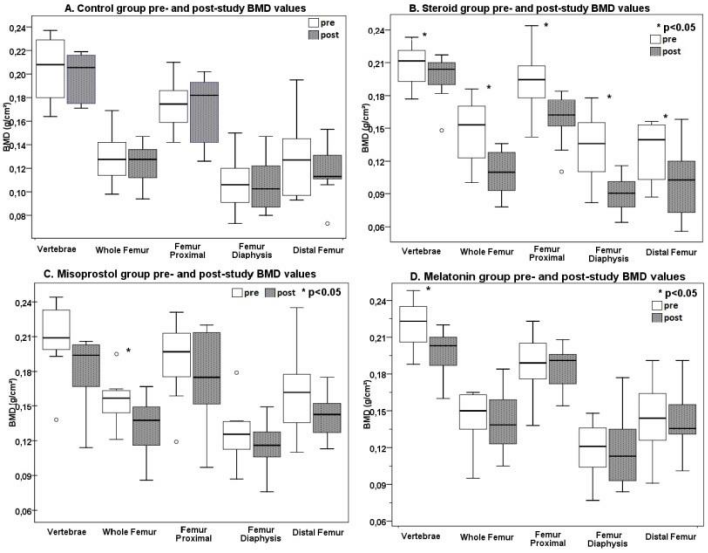

Figure 2: Pre- and post-study BMD alterations of vertebrae and femur regions. A: Control Group, B: Steroid Group, C: Misoprostol Group, D: Melatonin Group. * Significant difference between pre- and post-study BMD

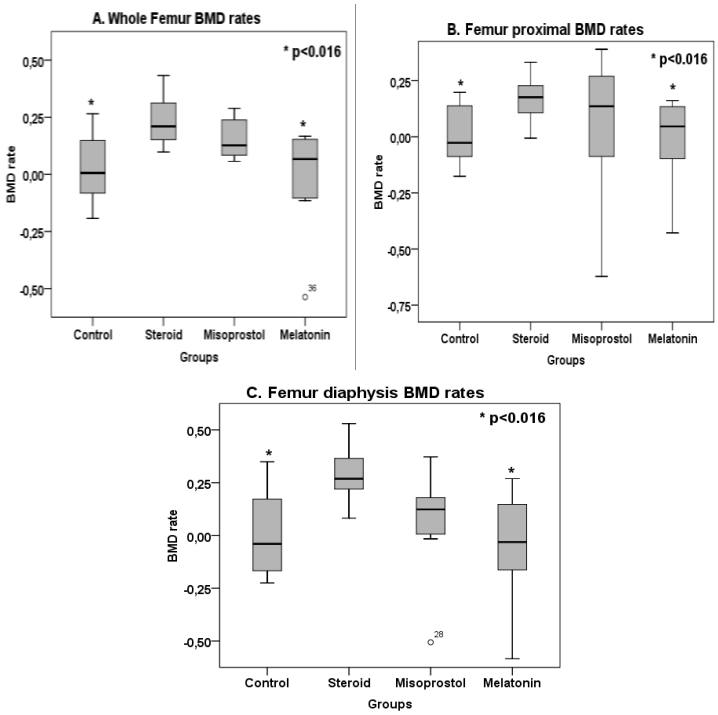

Figure 3: BMD rates of groups in femur regions that are showing significant differences in intergroup comparisons. A: Whole femur, B: Femur proximal, C: Femur diaphysis, * Significant differences compared to steroid group

Table 1: Weight and Bone Mineral Density variables in all groups before and after treatments

Groups	Variables	Before Median (Min-Max)	After Median (Min-Max)	*P*-value
Control (n=10)	Weight (gr)	150.0 (105-200)	149 (89-188)	0.443
	Vertebrae BMD (gr/cm²)	0.208 (0.164-0.237)	0.205 (0.171-0.219)	0.169
	Whole femur BMD (gr/cm²)	0.127 (0.098-0.169)	0.127 (0.094-0.147)	0.646
	Femur proximal BMD (gr/cm²)	0.174 (0.142-0.210)	0.182 (0.126-0.202)	0.906
	Femur diaphysis BMD (gr/cm²)	0.106 (0.073-0.150)	0.102 (0.080-0.147)	0.959
	Distal femur BMD (gr/cm²)	0.127 (0.093-0.195)	0.113 (0.073-0.153)	0.241
Steroid (n=10)	Weight (gr)	144 (83-165)	153 (99-181)	0.066
	Vertebrae BMD (gr/cm²)	0.211 (0.177-0.233)	0.204 (0.148-0.217)	0.011*
	Whole Femur BMD (gr/cm²)	0.153 (0.093-0.186)	0.109 (0.078-0.136)	0.005*
	Femur Proximal BMD (gr/cm²)	0.194 (0.142-0.244)	0.162 (0.110-0.184)	0.007*
	Femur Diaphysis BMD (gr/cm²)	0.136 (0.082-0.178)	0.090 (0.064-0.116)	0.005*
	Distal Femur BMD (gr/cm²)	0.139 (0.073-0.156)	0.102 (0.056-0.158)	0.013*
Misoprostol (n=8)	Weight (gr)	142.5 (121-163)	143.5 (115-164)	0.400
	Vertebrae BMD (gr/cm²)	0.209 (0.138-0.244)	0.194 (0.114-0.206)	0.093
	Whole Femur BMD (gr/cm²)	0.157 (0.121-0.195)	0.37 (0.086-0.167)	0.012*
	Femur Proximal BMD (gr/cm²)	0.197 (0.119-0.231)	0.175 (0.097-0.220)	0.401
	Femur Diaphysis BMD (gr/cm²)	0.125 (0.087-0.179)	0.116 (0.076-0.149)	0.161
	Distal Femur BMD (gr/cm²)	0.162 (0.101-0.235)	0.142 (0.113-0.175)	0.123
Melatonin (n=10)	Weight (gr)	143.5 (115-163)	140.5 (100-185)	0.445
	Vertebrae BMD (gr/cm²)	0.223 (0.188-0.248)	0.203 (0.160-0.220)	0.009*
	Whole Femur BMD (gr/cm²)	0.150 (0.095-0.165)	0.138 (0.105-0.184)	0.386
	Femur Proximal BMD (gr/cm²)	0.189 (0.138-0.223)	0.191 (0.154-0.208)	0.445
	Femur Diaphysis BMD (gr/cm²)	0.121 (0.077-0.148)	0.113 (0.084-0.177)	1.000
	Distal Femur BMD (gr/cm²)	0.144 (0.091-0.191)	0.135 (0.101-0.191)	0.483

P-values were determined by Wilcoxon Ranks test. * $P<0.05$

Table 2: BMD rates and intergroup comparison

Bone Regions	Groups	BMD rate Median (Min-Max)	*P*-value
Vertebrae	Control (n=10)	0.039 (-0.117- 0.101)	0.104
	Steroid (n=10)	0.051 (-0.005-0.164)	
	Misoprostol (n=8)	0.129 (-0.362-0.409)	
	Melatonin (n=10)	0.106 (-0.019-0.217)	
Whole Femur	Control (n=10)	0.006 (-0.193-0.265)	0.005*
	Steroid (n=10)	0.210 (0.098-0.432)	
	Misoprostol (n=8)	0.127 (0.057-0.289)	
	Melatonin (n=10)	0.067 (-0.537-0.167)	
Femur Proximal	Control (n=10)	-0.027 (-0.176-0.198)	0.048*
	Steroid (n=10)	0.176 (-0.006-0.332)	
	Misoprostol (n=8)	0.136 (-0.622-0.390)	
	Melatonin (n=10)	0.046 (-0.428-0.161)	
Femur Diaphysis	Control (n=10)	-0.040 (-0.225-0.349)	0.005*
	Steroid (n=10)	0.269 (0.082-0.530)	
	Misoprostol (n=8)	0.124 (-0.506-0.372)	
	Melatonin (n=10)	-0.032 (-0.584-0.269)	
Distal Femur	Control (n=10)	0.074 (-0.577-0.456)	0.126
	Steroid (n=10)	0.251 (-0.103-0.585)	
	Misoprostol (n=8)	0.132 (-0.591-0.404)	
	Melatonin (n=10)	0.036 (-0.560-0.195)	

P-values were determined by Kruskal-Wallis Test.* $P<0.05$

Table 3: BMD rates Post-hoc Multiple Comparison

Bone Regions	Groups		Difference	*P*-value
Whole Femur	Control	Steroid	-0.204	0.007*
		Misoprostol	-0.121	0.051
		Melatonin	-0.061	0.880
	Steroid	Misoprostol	0.083	0.131
		Melatonin	0.143	0.002*
	Misoprostol	Melatonin	0.060	0.110
Femur Proximal	Control	Steroid	-0.203	0.010*
		Misoprostol	-0.163	0.248
		Melatonin	-0.073	0.821
	Steroid	Misoprostol	0.040	0.594
		Melatonin	0.130	0.013*
	Misoprostol	Melatonin	0.090	0.374
Femur Diaphysis	Control	Steroid	-0.309	0.003*
		Misoprostol	-0.164	0.328
		Melatonin	-0.008	0.940
	Steroid	Misoprostol	0.145	0.026
		Melatonin	0.301	0.002*
	Misoprostol	Melatonin	0.156	0.286

P-values were determined by Man-Whitney U test; * $P<0.016$

Discussion

Glucocorticoids are the most common cause of drug induced osteoporosis. In this study, we compared the bone densitometry values of vertebrae and femur interest regions in rats that were treated with misoprostol or melatonin concurrently with 10 mg/kg/day methyl prednisolone for 28 days. At the end of the study, BMD values were determined to be significantly decreased in the GIO group, which was treated with methyl prednisolone only, indicating osteoporosis. In misoprostol administered group, the decrease in BMD was significant only in whole femur region. In the melatonin treated group, BMD was significantly decreased only in the vertebral regions, representing the protective effects of these agents. The lack of statistical significant differences in the misoprostol group in R1, R3, R4 and R5 regions and in melatonin group in R2, R3, R4 and R5 regions suggest that misoprostol and melatonin prevent osteoporosis induced by glucocorticoids.

With their well-known anti-inflammatory and immune modulating effects, glucocorticoids are commonly used in clinical practice. However, alterations in bone metabolism are one of the main adverse effects associated with glucocorticoid treatment in especially prolonged treatment modalities, leading to bone fragility and fractures. The main mechanism in GIO is the impaired osteogenesis. Glucocorticoids directly inhibit cellular proliferation and differentiation of osteoblasts and their maturation and activity [13,14]. Decreased bone formation is accompanied by an increase in bone resorption in prolonged usage of glucocorticoids. At a molecular level, glucocorticoids upregulate peroxisome proliferator-activated receptor gamma receptor 2 (PPARγ2) and alter the functions of Wnt/β-catenin signaling pathway which in turn favors the differentiation of pluripotent precursor cells to adipocytes instead of osteoblasts causing a decrease in osteoblast production. On the other hand, glucocorticoids also directly upregulate bone resorption by increasing the macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) production and decreasing osteoprotegerin formation by osteocytes. Reduced physical activity, increased renal and intestinal losses of calcium and reduced production of growth hormone and insulin-like growth factor 1 (IGF1) are the other factors that also contribute to GIO [15,16].

In this study, osteoporosis was induced in rats with a previously reported method [17]. Briefly, all rats except controls

were injected subcutaneously (sc) with methylprednisolone 10 mg/kg/day for 4 weeks. In the steroid group, BMD values of lombar vertebrae and different femur sites were determined to be statistically significantly decreased compared with the control group, showing the development of osteoporosis. In addition, alterations in the BMD values of steroid group were statistically significantly higher than the control group, as expected.

Misoprostol is an analogue of PGE1 that is mainly used in gastrointestinal system diseases to inhibit gastric acid secretion or in gynecological diseases for induction of labor and cervical dilatation. Although the anabolic effects of misoprostol on bone turnover have been known for years, the data about the effects of misoprostol on osteoporosis is limited in the literature [18,19]. Misoprostol was shown to induce epidermal growth factor expression and production by annulus cells [18]. With long-term misoprostol treatment, an increase in bone turnover, possibly with a net anabolic effect, was reported by Raisz et al. [20] in an experimental model. In another experimental study, Sonmez et al reported that 60 days of misoprostol treatment (100 and 200 micrograms/kg/day) restored bone loss in the lumbar spine of rats who had bilateral oophorectomy in a dose-dependent manner [21]. Ahmet-Camcioglu et al. [22] investigated the effects of misoprostol on BMD in 60 oophorectomized rats and determined that misoprostol could prevent bone loss only in the vertebrae. Misoprostol is inexpensive, easy to access, stored at room temperature and is preferred due to its minimal side effects. During our study 2 rats from misoprostol group were lost because of gastrointestinal system hemorrhage due to damage done by oral feeding cannula. We applied 200 μg/day dosed misoprostol orally for 28 days. When the misoprostol treated group was compared with the steroid group, the decrease of the BMD on all interest sites were observed to be less. However, this decrease which is indicative of osteoporosis was significant only in the whole femur region in the misoprostol group. As a result misoprostol was found to protect the bones from GIO except whole femur region.

Anabolic effects of melatonin on bone remodeling have been stated before. Melatonin induces the differentiation of primary osteoblasts, increases procollagen type I c-peptide expression in normal bone cells, stimulates type 1 collagen synthesis and proliferation in bone cells and inhibits bone resorption and increases bone mass by decreasing RANKL-mediated osteoclast formation [23]. However, Sethi et al. [24] reported that, a continuous 21-day melatonin exposure was required to induce osteoblast differentiation from human mesenchymal stem cells to induce osteogenesis. Recently, Sharan et al. [25] determined that melatonin was a regulator of bone mass through MT2 receptors supporting the therapeutic role of melatonin in postmenopausal osteoporosis. Amstrup et al. [26] reported that a one-year melatonin treatment resulted in an increase in BMD at femur neck and in the spine in a dose-dependent manner. The dietary melatonin supplementation was defined to have beneficial effects against the age-related bone loss in old rats, improving the microstructure and biomechanical properties of bones in different studies [27]. Melatonin was also defined to improve estrogen deficiency-induced osteoporosis and impaired osteogenic differentiation through mediating the Wnt/β-catenin pathway [28]. Melatonin has anti-oxidant properties which may also be important in prevention of GIO [29]. In our study, when melatonin-treated group was compared with the steroid group, the decreases of BMD on all interest sites except vertebrae region were observed to be less. We determined that melatonin was effective in preventing BMD decreases in steroid treated rats. Consequently, melatonin protected bones from GIO except the vertebrae region.

Besides, there was a significant difference between the BMD values of groups showing the amount of BMD changes in the proximal femur, diaphyseal femur and whole femur regions. The BMD rates of the melatonin group were statistically significantly lower than the steroid group. However, there were no significant differences between BMD values of the misoprostol, steroid and control groups in related bone regions. In that aspect, we can suggest that concurrent melatonin administration may be more effective in preventing GIO than misoprostol.

The most effective method of diagnosis and treatment follow-up for osteoporosis is DEXA [30]. In our study we used DEXA to show BMD measurements as it is easy to apply, less costly, and it can give simultaneous measurements for peripheral and spinal bone structures with less radiation exposure in a shorter time. Some histological parameters were assessed in almost all of the above-mentioned studies, but when human applications are considered we believe they would not be practical.

Limitations

There are some limitations of this study. First, we did not analyze biochemical markers or histological evaluation of bone formation and destruction in rats. If these blood and urine tests or tissue analyses were available, we could have a more detailed data to understand the pathogenesis of GIO and protective effects of misoprostol and melatonin. The second point was that the lumbar region and femur were not sampled for histomorphometric investigations.

Conclusion

Positive effects of misoprostol and melatonin on bone metabolism in rats with GIO were determined in this study. Misoprostol and melatonin seem to be potential agents that can be used in the prevention of osteoporosis in the near future because they are inexpensive, cost-effective and patient-compliant with fewer side effects. Larger prospective, clinical studies are required to determine the exact role of these molecules on prevention of GIO.

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