Original Article

Effects of Glucocorticoid on Bone Metabolism Markers and Bone Mineral Density in Rats.

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Abstract:

Statement of Problem: Glucocorticoid-induced osteoporosis is characterized by a decrease in osteoblast numbers and a marked impairment of new bone formation.

Purpose: The ain of present study was to evaluate the effect of methylprednisolone acetate on bone metabolism and bone mineral density in rats.

Materials and Methods: Eighteen male Sprague Dawley rats (8 weeks old, weighting 180 gm) were randomly divided into three groups: Group A (n=6), was a baseline control consisting of normal animals. Group B (n=6), was treated only by normal saline injection (0.9%) and group C (n=6), injected Methylprednisolone acetate (0.2 mg/kg/s.c. 3 times/week for 4 weeks). Changes in biochemical agents of serum calcium were evaluated by measuring acid phosphatase and osteocalcin, before and after treatment. Bone mineral density (BMD) of the lumbar vertebrae was also measured by dual energy x-ray absorptiometry (DEXA).

Results: The results showed that, serum calcium levels were not affected by methylprednisolone acetate ($p>0.05$), but acid phosphatase levels of serum increased significantly ($p\leq0.05$). In addition, the serum osteocalcin levels and bone mineral density of lumbar vertebrae decreased significantly ($p\leq0.05$) in the methylprednisolone acetate-treated group as compared to the other groups.

Conclusions: The findings indicate that administration of methylprednisolone acetate decreases bone formation and increases bone resorption in the lumbar vertebrae.

Key words: Glucocorticoid; Osteoporosis; Bone metabolism markers; BMD

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INTRODUCTION

Glucocorticoid-induced osteoporosis has been recognized since 1932 [1], but its exact mechanism has not been fully elucidated. Following factors have been proposed to be involved in the pathogenesis of osteopenia: decreased of intestinal calcium absorption [2-4] and decreased renal tubular calcium reabsorption with a consequent increase of the urinary calcium level [5,6]. Diminished

calcium absorption and increase of its excretion could lead to secondary hyperparathyroidism [7,8]. In addition, in steroid-induced osteoporosis, gonadotropine and sex steroid production is down regulated [9] and synthesis of local osteoblast growth factors, such as insulin-like growth factor (IGF)-1, (IGF)-2 and transforming growth factor-B (TGF-B) is repressed [10]. However, the principal cause of decreased bone mass in steroid-induced osteoporosis is a marked reduction in osteoblast number and function, resulting in reduced new bone formation. The differentiation of osteoblasts is controlled by growth factors and cytokines [11-14]. Low doses of glucocorticoids are essential for normal osteoblast function and inducing osteoblast differentiation by increasing the expression of mature bone markers, such as alkaline phosphatase and osteocalcin [15]. However, the high doses used to achieve clinical immune suppression, dramatically reduce the number and function of mature osteoblasts, with a decrease in osteocalcin. It has been shown that glucocorticoid-treated mice exhibit a threefold increase in apoptosis of vertebral osteoblasts and that apoptosis is detected in as many as 28% of the osteocytes in the metaphyseal cortical bone [14]. Bone loss with resulting fractures is one of the most dramatic outcomes of steroid therapy. Steroid-induced osteoporosis is produced by the imbalance between bone formation and bone resorption. Most patients do not develop osteoporosis at the beginning of glucocorticoid therapy and therefore it is important to identify people who are at the risk of osteoporosis by closer follow up. Evaluation of urine and serum calcium concentration and bone mineral density is helpful in assessing calcium balance [16]. Serum osteocalcin has been commonly employed clinically as a bone formation marker and has been used to assess the effects of experimental agents on bone metabolism in several animal models [17].

Dual energy X-ray absorptiometry (DEXA) has been proven to be a precise and reliable method for measuring bone mineral density (BMD) in rat models, both in vivo and in vitro [18,19]. The aim of the present study was to evaluate the effect of methylprednisolone acetate on bone metabolism in rats by use of biochemical markers and bone mineral densitometry.

MATERIALS AND METHODS

Glucocorticoid treatment of animals: Eighteen male Sprague Dawley rats (8 weeks old, weighting 180g) were used in this study. The animals were kept in plastic cages, under standard laboratory conditions with a constant temperature of 24 degrees centigrade and a 12-h light, 12-h dark cycle. All rats were maintained on a standard diet of laboratory rat chow containing 0.75% calcium, 0.6% phosphorus, 500 IU /kg vitamin D3 and had free access to tap water. They were kept on this diet throughout the study period. The animals were divided randomly into three groups: Six rats served as the baseline control (group A), Six rats as sham (group B), receiving normal saline by subcutaneous injection (0.9%, l00 microliter/100g body weight, 3 times/week for 4 weeks), and finally the remaining 6 rats (group C), were injected Methylprednisolone acetate (Red Crescent, Iran) subcutaneously (0.2 mg/kg, 3 times/week for 4 weeks).

In order to assess bone metabolic markers, blood was drawn before the injections by puncturing the orbital sinus under diethyl ether anesthesia, and the same procedure was repeated before scarifying the animals. The blood samples were immediately centrifuged and serum samples were stored at -70 degrees centigrade until assays were performed. All rats were scarified by overdosing the diethyl ether at the end of the 4 week period. For evaluation of the lumbar vertebrae bone mass, mineral densitometry was performed.

Determination of Bone Metabolic Markers: Total serum calcium and acid phosphatase were determined by a spectrophotometer using commercially available test kits (Ziestchem diagnostic, Tehran, Iran) [20]. Serum osteocalcin was assessed by enzyme immunoassay (DRG Instrument GmbH, Germany).

Bone Mass Measurement: The bone mineral content of lumbar vertebrae was measured by dual energy X-ray absorptiometry (DEXA) using the Norlaand small subject, resolution 0.5 X 0.5 mm, speed 60mm/s, Host scanner 3.2,3.2 and 1.1. Bone mineral density (BMD) was expressed as gram of mineral per unit area of bone (g/cm2).

Statistical Analysis: The obtained bio-chemical markers and BMD measurements were analyzed using the one way analysis of variance (ANOVA). Duncan and Dunnett tests were utilized to compare the mean values between the three groups. Statistical significance was set at a p value of ≤0.05.

RESULTS

Effects of methylprednisolone acetate on serum bone biochemical markers: Table 1, demonstrates serum calcium levels in groups A, B and C before and after treatment. A statistically significant difference (P<0.05) was not observed in serum calcium levels among the groups before and after treatment. In addition, serum osteocalcin concentration means did not show a statistically significant difference between the three groups before the treatments. After treatment, the concentration of serum osteocalcin (a parameter of bone formation) was significantly lower (P≤0.05) in group C, in comparison to the control groups.

Table 1 also shows the levels of serum acid phosphatase in all three groups before and after treatment. A statistically significant difference was not observed between groups before treatment (P>0.05); but, after treatment, the level of serum acid phosphatase increased significantly (P≤0.05) in the glucocorticoid treated rats as compared to the other groups. Administration of methylprednisolone acetate decreased the level of serum osteocalcin (P≤0.05) and increased serum acid phosphatase (P≤0.05).

Effect of methylprednisolon acetate on lumbar vertebrae BMD: After 4 weeks of glucocorticoid treatment, bone mineral content and bone mineral density (g/cm2) decreased significantly (P≤0.05) in the glucocorticoid treated animals as compared to the other groups (Table 1 and Fig. 1A,B).

DISCUSSION

The present study was designed to evaluate the effect of glucocorticoides on bone metabolism in rats by using biochemical markers and measuring bone mineral density. According to our results, administration of 0.2 mg/kg methylprednisolone acetate causes bone loss in rats. This is in agreement with previous findings [16,21] which have shown that administration of glucocorticoid, leads to a

Table I: Serum biochemical markers and bone mineral density (BMD) of lumbar vertebra in studied groups. Ca=Calcium, AcP=Acid Phosphatase, Osc=Osteocalcin, MPA=Metyl Prednisolone Acetate, BMD=Bone Mineral Density, A=After treatment, B=Beafor treatment

Elements	Ca(mg/dl)B	Ca(mg/dl)A	AcP(tu/l)B	AcP(tu/l)A	Osc(ng/ml)A	BMD(g/cm²)A
Control	8.87 (0.97)	8.94 (0.95)	26.33 (7.36)	30.44 (6.09)	3.49 (1.84)	0.1362 (5.94E0.4)
Placebo	8.82 (0.06)	8.60 (50.96)	26.83 (6.07)	33.35 (6.34)	2.22 (0.62)	0.1320 (1.19E0.2)
MPA	8.76 (0.51)	8.23 (0.55)	35.81 (10.03)	55.10 (14.73)	1.50 (0.59)	0.1201 (7.57E0.3)

Values are average (standard deviation)

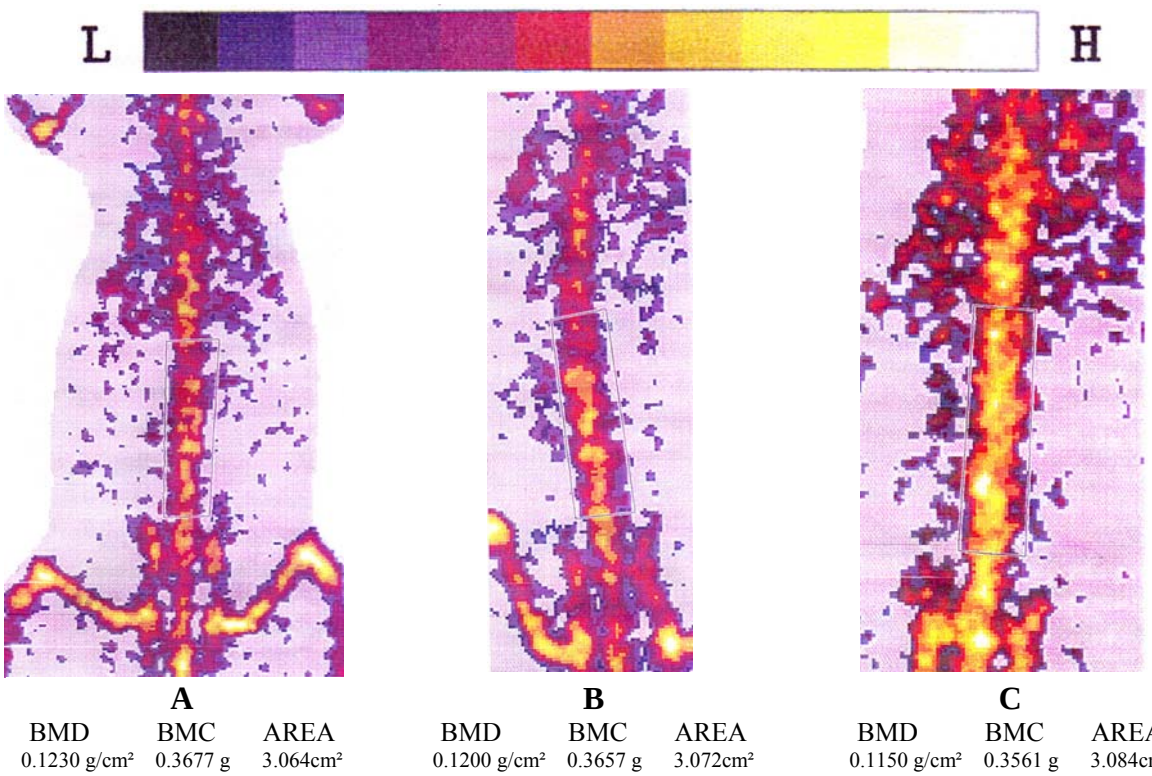

Fig.1: Bone mineral densities of lumbar vertebra in control (A), Placebo (B), and Methyl Prednisolone acetate (C) groups.

decrease in bone mass.

The rat model of glucocorticoid-induced osteoporosis has been well documented, although rats have been reported to be relatively resistant to glucocorticoid-induced bone damage, both in vivo and in vitro [21]. However in several in vivo studies, marked inhibition of bone formation [22] and decrease in BMD [23] and cortical strength [23] have been reported. Prednisolone treatment, increased the 24-h renal calcium excretion by 45% and induced a small increase in serum PTH without affecting serum $1,25(OH)_2$ D_3 and serum calcium [24]. Other studies using short courses of glucocoricoids have also shown increased renal calcium excretion and unaltered serum calcium levels [25]. Glucocorticoid administration could decrease intestinal absorption of calcium [3] and intestinal hyperabsorption is unlikely to be the cause of the urinary calcium excretion found in prednisolone-treated cases [24]. More likely glucocorticoids may enhance renal calcium excretion directly by altering the tubular reabsorption [3,26] or indirectly by an increased filtered calcium load secondary to rapidly decreasing bone formation or increasing bone resorption [26]. On the other hand the unaltered serum calcium may be caused by a concomitant net increment of bone resorption. The results of the present study are in accordance with these findings. Tartrate-resistant acid phosphatase (TRAP) is a lysosomal hydrolyser, which has been shown to be released from osteoclasts during bone resorption. Acid phosphatase is present in bone, spleen, prostate, erythrocytes and platelets. In serum, only bone and erythrocyte isoenzymes of acid phosphatase are insensitive to tartrate, Therefore in the absence of hemolysis, the activity of this isoenzyme, TRAP, can be used as an index of osteoclastic

activity, i,e bone resorption [27]. In the present study serum acid phosphatase was increased in the experimental group as compared to the other groups. Some researchers reported that osteocalcin is a noncollagenous matrix protein produced by osteoblasts and serum osteocalcin correlates with the rate of bone mineralization [28]. Other investigators stated that gluco-corticoids inhibit bone formation both by a direct inhibition of osteoblast function and by a suppression of the proliferation and differentiation of osteoblast precursor cells [7]. Prednisolone treatment has been shown to inhibited bone formation as estimated by a decrease in osteocalcin levels [25,29]. In the present study, serum osteocalcin levels decreased in the experimental groups at the end of the 4th week.

CONCLUSION

Utilizing biochemical markers and Bone Mineral Densitometry (BMD), this study confirmed that 0.02mg/kg methylprednisolone acetate administration (3 times a week for 4 weeks) could induce osteoporosis in rats.

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اثر گلوکو کورتیکوئید بر نشانگرهای متابولیسم استخوان و چگالی معدنی استخوان Rat

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چکیده

بیان مسأله: استئوپروز ناشی از گلوکو کورتیکوئیدها به صورت کاهش در تعداد استئوبلاست‌ها و نقایص مشخص ساختمانی در استخوانهای جدید مشخص می‌شود.

هدف: مطالعه حاضر با هدف تعیین اثر متیل پردنیزولون استات بر متابولیسم و چگالی معدنی استخوان Rat انجام شد.

روش تحقیق: تعداد ۱۸ Rat نر از نژاد Sprague Dawley (با سن ۸ هفته و وزن حدود ۱۸۰ گرم) به طور تصادفی به سه گروه ۶ تایی تقسیم شدند. گروه A (شاهد) شامل نمونه‌های سالم حیوانی بود. در گروه B تزریق نرمال‌سالین انجام شد و در گروه C نیز متیل‌پردنیزولون زیرپوستی به مدت ۴ هفته و ۳ بار در هفته به میزان ۰/۲ میلیگرم به ازای هر کیلوگرم وزن حیوان تزریق شد. تغییرات عوامل بیوشیمیایی کلسیم سرم، اسید فسفاتاز و استئوکلسین با اندازه‌گیری قبل و بعد از درمان مقایسه شد. چگالی معدنی استخوان (BMD) مهره‌های کمری با استفاده از روش DEXA (Dual Energy X ray Absorptiometry) تعیین شد.

یافته‌ها: سطح کلسیم سرم، تحت تأثیر متیل پردنیزولون استات تغییر نیافت ($P>0/05$)، اما اسید فسفاتاز سرم به طور معنی‌داری افزایش نشان داد ($P<0/05$). سطح استئوکلسین سرمی و چگالی معدنی استخوان در ناحیه مهره‌های کمری به طور معنی‌داری در گروه متیل پردنیزولون استات کاهش یافت ($P<0/05$).

نتیجه‌گیری: بر اساس نتایج حاصل از این تحقیق می‌توان اذعان کرد که تجویز متیل پردنیزولون استات، سبب کاهش شکل‌گیری استخوان و افزایش تحلیل استخوان مهره‌های کمری می‌شود.

واژه‌های کلیدی: گلوکو کورتیکوئید؛ استئوپروز؛ نشانگرهای متابولیسم استخوان؛ چگالی معدنی استخوان

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