Prevention of Methylprednisolone Acetate-Induced Osteoporosis with Calcium Administration in Rat Model

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Received: 22 Jul. 2007; Received in revised form: 3 Oct. 2007; Accepted: 20 Jan. 2008

Abstract- Glucocorticoid steroids are widely used as anti-inflammatory and immunosuppressive medications and are well known to induce osteoporosis. In Present study 24 rats were randomly divided into four groups (n=6): Group A (control), Group B (sham)that was treated only by normal saline for 1 month.Group C that was treated by methylprednisolone acetate alone (0.2 mg/kg) for 1 month. Group D that was treated by methylprednisolone acetate (0.2 mg/kg) and oral calcium supplementation (15 mg/kg) for 1 month. Changes in concentration of bone metabolic markers such as osteocalcine, acid phosphatase and calcium were evaluated before and after treatment. Bone mineral density (BMD) of lumbar vertebrae was also measured by dual energy X ray absorptiometry (DEXA). The results showed that concentration mean of serum acid phosphatase was increased significantly ($P \leq 0.05$) in C and D groups in compared to A and B groups. The concentration mean of serum osteocalcine in group C was decreased significantly ($P \leq 0.05$) in comparison to A and B groups but increased significantly in the group D in comparison to group C. The concentration mean of serum calcium was decreased significantly ($P \leq 0.05$) in C and D groups in compared to A and B groups. The bone mineral density (g/cm^2) was decreased significantly ($P \leq 0.05$) in group C in compared to A and B groups. This increased significantly in group D in compared to group C. These results are compatible with the view that low doses of methylprednisolone acetate decreases bone formation and increase bone resorption in the lumbar vertebrae of rats. Calcium administration decreased effects of methylprednisolone.

Acta Medica Iranica 2009; 47(4): 251-257.

Key words: Methylprednisolone acetate, osteoporosis, bone metabolism markers (BMM) and rat

Introduction

Osteoporosis is a major complication in patients who require chronic glucocorticoid treatment (1). The glucocorticoid-induced bone loss mainly results from depressed osteoblastic activity and differentiation, but many pathways contribute to the skeletal morbidity: enhanced osteoclastic activity, reduced intestinal calcium absorption, increased renal calcium excretion, disturbances in vitamin D and gonadal hormones metabolism and increased apoptosis of osteoblasts and osteocytes (2-5). The lumbar spine and proximal part of femur are particularly vulnerable to osteoporosis and related fractures caused by glucocorticodis (6). The bone density, which defines the degree of osteoporosis and the fracture risk, is generally determined by dual-energy X-ray absorptiometry (DEXA) (7). The main determinants of peak bone density are genetic, but there is some evidence that calcium intake and exercise also make a contribution (8). In addition there is surprisingly weak evidence on which to support clear recommendations about low doses of glucocorticois ($\leq$ 10 mg prednisolone equivalent per day) are essential for normal osteoblasts function, inducing osteoblasts differentiation by increased expression of mature bone markers, such as alkaline phosphatase and osteocalcine (9). Osteoporosis is largely preventable due to advances in our understanding of its causes and the availability of effective therapeutic interventions (10). Prevention of osteoporosis should be considered even when the

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glucocorticoid dose is intermittent or low (prednisolone < 5 mg/d or equivalent) (11). Optimal prevention and treatment of osteoporosis requires supplementation with calcium and vitamin D, particularly if dietary intake is in adequate (7). Most patients require calcium supplementation to meet the recommended daily intake of 1000 to 1500 mg/d (7, 11) and those at risk also may require vitamin D supplements (800 IU/d) (11). Although calcium and vitamin D and its derivatives have only weak antiresorptive effects compared with other pharmacological agents deficiency of either will compromise bone strength (12). However, there are discrepancies in the results of in vivo studies in man and animals. While high doses or long-term glucocorticoid therapy cause bone resorption and decrease bone mineral density (13, 14) other studies demonstrated that low-dose treatment increased bone formation (15, 16). Whether administration of low-dose glucocorticoids can decrease bone loss in glucocorticoids users has not been convincingly demonstrated so far (17). The present study was designed to evaluate the prevention effect of bone loss with calcium as an anti-resorptive agent in methylprednisolone acetate induced osteoprosis as a member of glucocorticoid family by using biochemical markers (calcium, osteocalcine, acid phosphatase) and Bone Mineral Density (BMD) in rat model.

Patients and Methods

In Present studies 24 male Sprague-Dawley rats (180-200gr) were purchased from Pasteur Institute (Iran). The rats were housed in cages in a controlled atmosphere room (temperature 24^{o}c, 12 h light: dark cycle). The animals fed from a standard rat chow containing 0.75 % calcium, 0.6 % phosphorus, 500 IU/kg vitamin D_3 (pars animals food) and tab water. They were kept on this diet through the study. Twenty four rats were randomly divided into four groups (n = 6). Group A, was a base line control or normal animal. Group B (sham), treated only by normal saline (0.9 %, 100 micro liter/100 gr. Body weight) injection subcutaneously (3 times weeks for 1 month). Group C, treated by methylprednisolone acetate alone (0.2 mg/kg) subcutaneously injection (3 times/week for 1 month). Group D, treated by methylprednisolone acetate (0.2 mg/kg) subcutaneously injection (3 times/week for 1 month) and 15 mg/kg oral calcium supplementation for 1 month.

Determination of bone metabolic markers

In order to assess bone metabolic markers, blood was drawn by puncturing of orbital sinus before and after performed the protocol under diethyl/ether anesthesia. The blood samples were immediately centrifuged and serum samples were stored at-70 centigrade degree until assayed. All rats were sacrified by overdosing chloroform at the end of 4 week period. Total calcium and acid phosphate concentration in serum were determined by spectrophotometery using commercially available test kit (Ziestchem diagnostic, Tehran, Iran) (11, 18). Also osteocalcine concentration in serum was determined by enzyme immuno assay (DRG instrument GmbH, Germany) before and after treatment in all groups.

Bone mineral densitometry (BMD)

BMD were performed for evaluation of bone mass in lumbar vertebrae (19). 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 600 mm/s, Host scanner 3.2, 3.2 and 1.1). The bone mineral density (BMD) was expressed as a gram of mineral per unit area of bone (g/cm^2).

Statistical analysis

In order to analysis biochemical markers and BMD measurement obtained in rats, one way analysis of variance (ANOVA), Duncan and Dunnett tests used to compare the means values between groups. A value of $P < 0.05$ was considered statically significant.

Results

Effect of methylprednisolone acetate and calcium supplementation on bone metabolic markers was evaluated. As the figure 1 shows, the concentration mean of serum acid phosphatase, a parameter of bone resorption, was increased significantly ($P \leq 0.05$) in group C in compared to A and B groups. This mean was increased significantly ($P < 0.05$) in group D in compared to A and B groups, but there was no significant difference between group C and D.

The concentration mean of serum osteocalcine, a parameter of bone formation, in group C was decreased significantly ($P \leq 0.05$) in comparison to A and B groups. In group D, concentration mean of serum osteocalcine was increased significantly ($P \leq 0.05$) in comparison with group C but this difference in comparison with A and B groups were not significant (Figure 2).

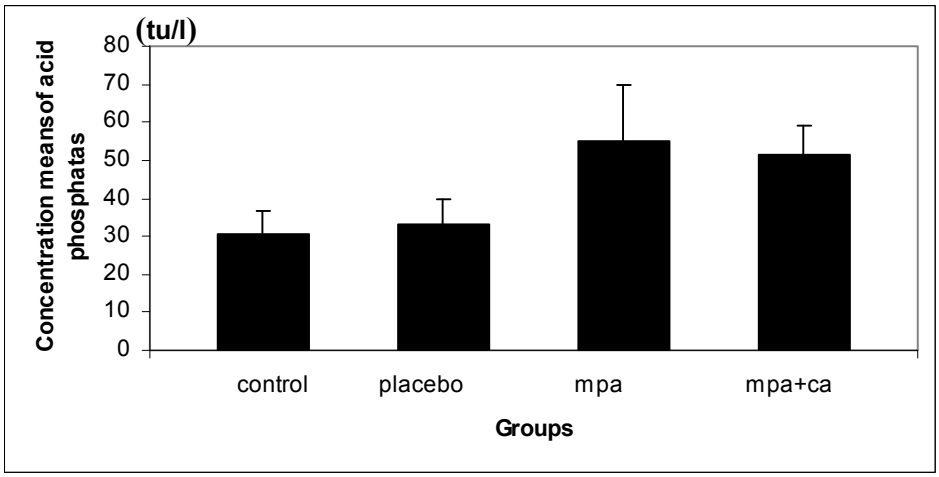

Figure 1. Effect of 0.2 mg/kg of methylprednisolone acetate with or without 15mg/kg of supplemental calcium for 1 month on concentration of serum acid phosphatase of 24 male Sprague-Dawley rats. Values are mean ± SE. Significant difference at $P < 0.05$. mpa = methylprednisolone acetate, mpa + ca = methylprednisolone acetate + calcium.

The concentration mean of serum calcium in group D in comparison to other groups were not significant (Figure 3). Effect of methylprednisolone acetate and Calcium supplementation on BMD of lumbar vertebrae was evaluated too. The bone mineral density (g/Cm^2) was decreased significantly ($P \leq 0.05$) in group C in compared to A and B groups but increased significantly in compared to the group D (Table 1) and (Figure 4).

Table 1. Effect of 0.2 mg/kg of methylprednisolone acetate with or without 15 mg/kg of supplemental calcium for 4weeks on bone mineral density (BMD) of 24 male Sprague-Dawley rats. Values are means ± SE. Significant difference at $P < 0.05$.

Group	Number	$\frac{BMD(g/cm^2)}{mean \pm SD}$
Control	6	0.1362 ± 5.94E-04
Placebo	6	0.1320 ± 1.19E-02
MPA	6	0.1201 ± 7.57E-03
MPA+Ca	6	0.1272 ± 5.46E-03

MPA=Methylprednisolone acetate, CA=Calcium

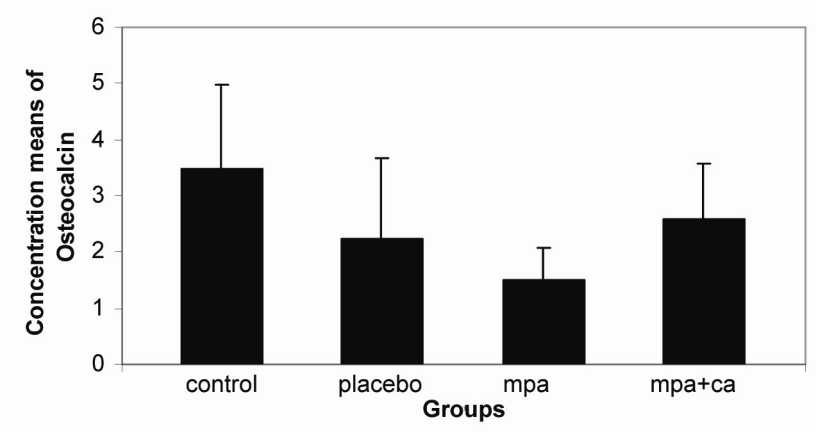

Figure 2. Effect of 0.2 mg/kg of methylprednisolone acetate with or without 15 mg/kg of supplemental calcium for 4weeks on concentration of serum osteocalcin of 24 male Sprague-Dawley rats. Values are means ± SE. Significant difference at P≤0.05. mpa=methylprednisolone acetate, mpa+ca=methylprednisolone acetate+calcium.

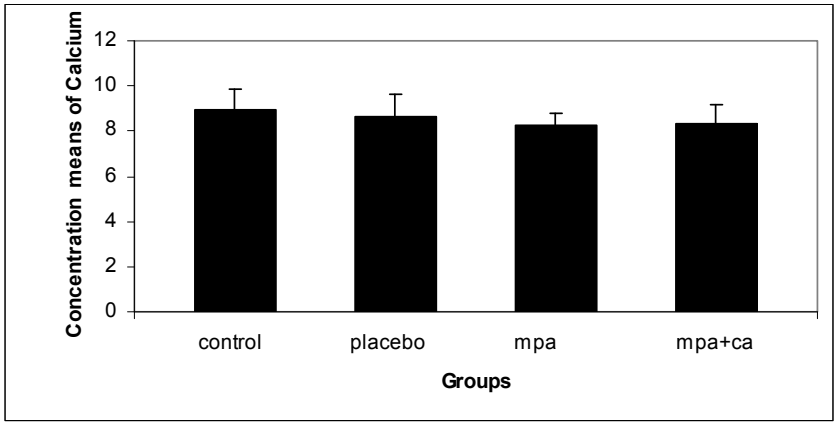

Figure 3. Effect of 0.2 mg/kg of methylprednisolone acetate with or without 15mg/kg of supplemental calcium for 4weeks on concentration of serum calcium of 24 male Sprague-Dawley rats. Values are means ± SE. Significant difference at $P < 0.05$.

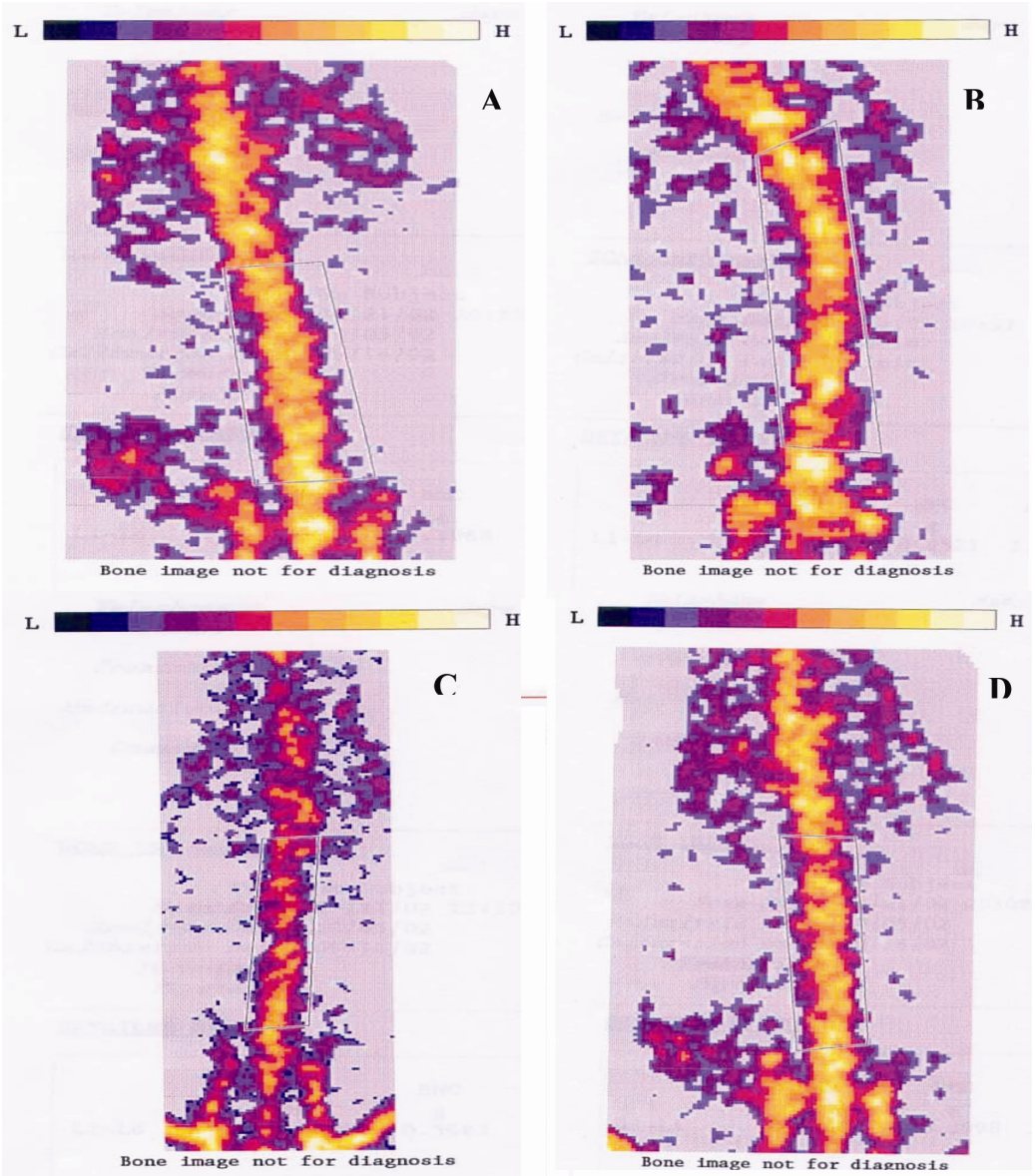

Figure 4. Bone mineral densities of lumbar vertebra was measured by dual energy X ray absorptiometry (DEXA) in control (A), sham (B), Methylprednisolone acetate (C), Methylprednisolone and oral calcium supplementation (D) groups.

Discussion

Our results demonstrated that administration of low dose methylprednisolone acetate cause bone loss in lumbar vertebrae of experimental group compare to control group. It has been shown that the administration of glucocorticoids induces an increase in bone resorption, which results in a deacrease in bone mineral density in various bones in human (20, 21). Even in animal experiments, it has been demonstrated that the administration of glucocorticoids induces a decrease in the total body bone mineral content and results in a decrease in BMD (22-24). It seems, Glucocorticoids impede osteoblast formation by inhibiting the release of cellular growth factors (4). Glucocorticoids have profound effects on mineral metabolism and skeletal function and can lead to the development of short statue and osteoporosis (25). The most significant effect of glucocorticoids in bone is an inhibition of bone formation (11). This is because of decrease in the number of osteoblasts and their function. The decrease in cell number is secondary to a decrease in osteoblastic cell replication and differentiation and an increase in the apoptosis of mature osteoblasts (5). Anyway, In contrast to our study some long-term studies showed improvement or even normalization of bone mineral density after treatment with low dose of methylprednisolone acetate (26-28). Although the Medical Research Council and Nuffield Foundation trials in the mid-1950s and mid-1960s suggested a possible disease-modifying role for low dose of glucocorticoids (25). It is difficult to interpret the results of these trials, however, because of the heterogeneity of the patient groups, the long duration of disease at the start of studies, confounding by indication and multiple concomitant therapies (26). The results of present study also showed that administration of low dose methylprednisolone acetate cause significant increase in bone resorption marker (acid phosphatate) and a moderate decrease in bone formation marker (osteocalcin) of experimental group compared to control group. Our findings confirmed that the loss of bone mineral density resulted primary from decreased bone formation which reported by some researchers (2, 16). Also, According to our results, administration low dose of methylprednisolone acetate with 15 mg/kg of calcium supplementation cause significantly increase in bone mineral density and osteocalcin concentration and did not give rise to any significant reduction in acid phosphatase compared to low dose of methylprednisolone acetate alone. As intestinal calcium absorption is impaired by glucocorticoid therapy, it seems logical to increase calcium intake in the diet or by supplementation (29, 30), suggesting that calcium alone is not sufficient to prevent rapid bone loss in patients starting high-dose glucocorticoids (31). Although our finding were contrary to previous findings in humans and animals (27, 28), they are consistent with the data from recently studies that showed increased bone mass or prevention of bone loss by calcium administration synchronize with glucocorticoids treatment (29-32). There are multiple mechanisms underlying the regulation of bone remodeling, and these involve not only the osteoblastic and osteoclastic cell lineages but also other factors (33, 34). Decreased calcium intake, impaired intestinal absorption of calcium due to aging or disease, as well as vitamin D deficiency can result in secondary hyperparathyroidism (35). The active hormonal form, 1,25 dihydroxy vitamin D (calcitriol), is not only necessary for optimal intestinal absorption of calcium and phosphorus, but also exerts a tonic inhibitory effect on parathyroid hormone (PTH) synthesis, so that there are dual pathways that can lead to secondary hyperparathyroidism (34, 35). Vitamin D deficiency and secondary hyperparathyroidism can contribute not only to accelerated bone loss and increasing fragility of bone but also to neuromuscular impairment that can increase the risk of falls (36). Clinical trials involving individuals follows prednisolone exposure at high risk for calcium and vitamin D deficiency indicate that supplementation of both can reverse secondary hyperparathyroidism, increase bone mass, decrease bone resorption, decrease fracture rates, and even decrease the frequency of falling (37). Results from the present study support the hypothesis similar to observation made by osteoporosis is characterized by low bone mineral density and a deterioratic the micro architecture of bone that increase its susceptibility to fracture, calcium and vitamin supplementation likely to decrease bone complications. Thus Bone loss can be minimizing through proper nutrition, calcium and vitamin supplementation (30). In conclusion, the present data suggest that Calcium administration in optimal condition decreased effects producing bone loss in lumbar vertebrae of rats resulted methylprednisolone acetate.

Acknowledgment

This project financially supported by research deputy of Tehran University of Medical Sciences. So, we would like to thank for their helps.

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