

PROTECTIVE EFFECT OF VITAMIN D3 IN METHYLPREDNISOLONE ACETATE (MPA) INDUCED LOSS OF BONE METABOLISM MARKERS AND BONE MINERAL DENSITY IN THE LUMBAR SPINE OF RAT

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Abstract- Although some vitamins have been shown to prevent glucocorticoids induced osteoporosis in short term, the magnitude of this effect remains to be clarified. The aim of this study was to evaluate protective effect of vitamin D3 on methylprednisolone acetate (MPA) induced osteoporosis in rats. Twenty-four male Sprague Dawley rats were randomly divided into four groups: group A (n = 6) was a baseline control or normal animals; group B (n = 6) was treated only by normal saline; group C (n = 6), was treated by MPA (0.2 mg/kg) subcutaneously for 4 weeks (3 times per a week) and finally group D (n = 6) were administered MPA similar to group C plus vitamin D3 (0.1 µg/kg dissolved in ethanol daily). Level of calcium, osteocalcin and acid phosphatase in serum were measured before and after treatment. Also, bone mineral density (BMD) of lumbar vertebrae was measured by dual energy X-ray absorptiometry. The results showed that the serum calcium level was unaffected by MPA in all groups before and after treatment, but the serum osteocalcin level and BMD of lumbar vertebrae were significantly ($P < 0.05$) decreased in group C compared with groups A and B. In group D serum osteocalcin level increased again significantly ($P < 0.05$) but increasing of BMD and bone mineral content were not significant. The findings indicate that using of vitamin D3 in MPA treated rats could increase bone formation and decrease bone resorption.

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INTRODUCTION

Glucocorticoid induced osteoporosis has been known since 1932 (1). The exact mechanism by which glucocorticoids cause bone-loss has been not fully elucidated. In accordance with other works (2-4), our previous study on methylprednisolone acetate induced osteoporosis showed loss of serum bone biochemical markers and bone mineral density in rat

(5).

It is generally accepted that low doses of glucocorticoids are essential for normal osteoblast function and inducing osteoblast differentiation by increasing expression of mature bone markers, such as alkaline phosphatase and osteocalcin (6). However, the high doses used to achieve clinical immune suppression dramatically reduce the number and function of mature osteoblasts, with a decrease in osteocalcin level. Glucocorticoid-treated mice show a threefold increase in apoptosis of vertebral osteoblasts and apoptosis was detected in as many as 28% of osteocytes from metaphyseal cortical bone (7).

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Osteoporosis is a painful, disabling and disfiguring process, especially affecting old women and who are being treated by glucocorticoids (8). Vitamin D and calcium deficiency also commonly occur in older individuals and in those who use glucocorticoids and live in nursing house, increasing the risk of osteoporosis and fractures (9, 10). Recently, Grados *et al.* reported that calcium and vitamin D supplements in elderly women with vitamin D deficiency could significantly increase median bone mineral density and similarly decrease median levels of main bone markers (11). On the other hand, other researchers say it is not possible to correct vitamin D deficiency with the nutritional vitamin D doses in postmenopausal women with decreased bone mineral density (12).

The major causes of vitamin D deficiency are poor nutrition, deprivation of sunlight with consequent decline in the synthesis of coetaneous vitamin D3 and decreased renal hydroxylation of 25(OH)D by the aging kidney (13, 14). The recommended diagnostic threshold and relationship to bone turnover markers and bone mineral density of the three vitamin D subgroups have been presented in Peacock's work (15). Measurement of urine and serum calcium concentration and bone mineral density is helpful in assessing calcium balance (16). Also, serum osteocalcin and alkaline phosphatase activity have been commonly employed clinically as bone formation markers and used to assess the effects of experimental agents on bone metabolism in several animal models (17).

Although some studies have been carried out on the effect of vitamin D3 or its metabolites on methylprednisolone acetate induced osteoporosis, the evaluation methods and protocols of these studies have not been the same. Dual energy X-ray absorptiometry (DEXA) has been proved to be a precise and reliable method for measuring bone mineral density in rat both *in vivo* and *in vitro* (18, 19). In the present study we decided to evaluate the vitamin D3 protective role on control of methylprednisolone acetate induced osteoporosis by measuring biochemical markers and bone mineral densitometry using DEXA in rat model.

MATERIALS AND METHODS

Twenty four male Sprague Dawley rats (8 week old and 180 g weight) used for this experiment. All experiments on animals were performed in accordance with UK legal requirements.

The animals were kept at 24 degree centigrade with a 12 h light and 12 h dark condition. The animals fed from a standard rat chow containing 0.75% calcium, 0.6% phosphorus, 500 IU/kg vitamin D3 (Pars animals' foods) and tap water and were kept on this diet through the study. The animals were divided randomly into four groups. Six rats served as baseline control (group A). Six rats served as sham (normal saline 0.9%, 100 µl/100 g body weight subcutaneously 3 times/week for 4 weeks (group B). Six rats were administered subcutaneously methylprednisolone acetate (0.2 mg/kg, 3 times/week for 4 weeks) as group C and finally six rats were administered methylprednisolone acetate similar to group C but also were treated by vitamin D3 (0.1 µg/kg dissolved in ethanol daily) as group D.

For examination of bone metabolic markers blood was taken by puncture of orbital sinus before and after performing the protocol under diethyl ether anesthesia. The blood samples immediately were centrifuged and serum samples were stored at -70 centigrade degrees until assayed. All rats were killed by overdose chloroform at the end of 4 weeks. For evaluation of bone mass in lumbar vertebrae bone mineral densitometry were performed.

Before and after treatment in all groups', total calcium and acid phosphatase in serum were determined by spectrophotometer using commercially available test kit (20-22). Also, osteocalcin in serum was determined by enzyme immunoassay (23). The bone mineral content of lumbar vertebrae was measured in all groups only after four weeks by DEXA using the Norland, small subject, resolution 0.5 × 0.5 mm, speed 60 mm/s, Host scanner 3.2, 3.2 and 1.1. The bone mineral density was expressed as gram of mineral per unit area of bone (gr/cm²).

One way analysis of variance (ANOVA), Duncan and Dunnett tests used to compare the means values between groups. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

Effect of vitamin D3 on serum bone biochemical markers in methylprednisolone acetate administrated animals:

The levels of serum calcium in groups A, B, C and D are presented in Table 1. There was no significant difference ($P > 0.05$) in serum calcium levels among the groups before and after treatment (Table 1). Also, as shown in table 1, mean of the serum calcium concentration in all groups did not have significant differences before treatment. But after treatment, the level of serum calcium in group C showed a statistically significant increase ($P < 0.05$).

Concentration of serum osteocalcin, as a parameter of bone formation, in group C was decreased significantly ($P < 0.05$) in comparison to control group, but in group D it increased to level of group C. These changes in serum osteocalcin level were significant ($P < 0.05$).

The levels of serum acid phosphatase in all groups before and after treatment are presented in table 1. There was no significant difference before treatment ($P > 0.05$). After treatment, the level of serum acid phosphatase was increased significantly ($P < 0.05$) in group C compared to groups A and B. In group D serum acid phosphatase level was decreased but this different was not significant ($P > 0.05$).

Effects of vitamin D3 on lumbar vertebrae bone mineral density:

After 4 weeks of methylprednisolone acetate administration, bone mineral content and bone mineral density (gr/cm^2) were decreased significantly ($P < 0.05$) in group C compared with groups A and B (Figure 1). Bone mineral content and bone mineral density (gr/cm^2) was increased, not significantly, in vitamin D3 treated group (Group D).

DISCUSSION

Bone tissue is a complex and metabolically active organ. The bone mineral is composed essentially of calcium and phosphate salts (9). They are essential for normal skeletal growth, maintenance of skeletal mechanical integrity and as pool for the extracellular calcium compartment (24). It has been demonstrated previously that the administration of excessive glucocorticoids induce a decrease in bone formation and increase in bone resorption which results in a decrease in bone mineral density in various bones of different animals (25-27). Also, vitamin D deficiency leads in adults to osteomalacia and in children to rickets, along with defective mineralization and increase in total alkaline phosphatase (28, 29). The present study was designed to evaluate the vitamin D3 protective effects on control and recovery of methylprednisolone acetate induced osteoporosis by evaluation of biochemical markers and bone mineral densitometry in vertebrae of rat.

In the present study, the administration of 0.2 mg/kg methylprednisolone acetate for 4 weeks produced bone loss in comparison to control groups (groups A and B) which is confirmed by decreasing of serum osteocalcin level and bone mineral density in vertebrae. These findings are in agreement with some (30) but opposite to other (25) works which reported an increase in the bone mineral density after methylprednisolone acetate administration. This difference may be resulted from methylprednisolone acetate treatment protocol, which was designed based on dose-dependency.

Present study demonstrated that treatment by vitamin D3 recovered bone resorption by increasing serum osteocalcin level. It is well known that administration of glucocorticoids decrease intestinal calcium absorption and increase urinary calcium excretion (31). Also, glucocorticoids inhibit collagen

Table 1. Comparison of bone metabolic markers within the experimental groups*

Groups	Calcium	Acid Phosphatase	Osteocalcin
Control (n = 6)	8.94 ± 0.95	30.44 ± 6.09	3.49 ± 1.48
Sham (n = 6)	8.65 ± 0.96	33.35 ± 6.34	2.22 ± 0.62
Methylprednisolone acetate (n = 6)	8.23 ± .55	55.10 ± 14.73	1.5 ± 0.59
Methylprednisolone acetate + D3 (n = 6)	8.35 ± 1.52	48.24 ± 16.87	2.70 ± 0.80

*Data are given as mean ± SD.

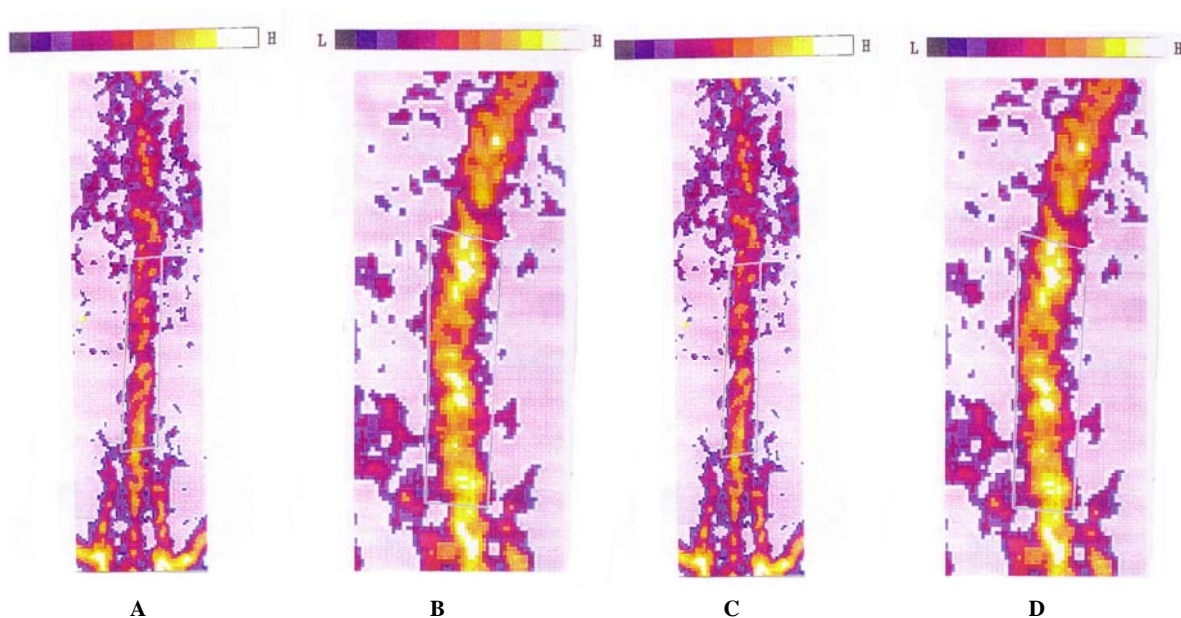


Fig. 1. Comparison of bone mineral density and bone mineral content of lumbar vertebrae. A: control (BMC = 0.4097 gr.; area= 3.052 cm²; BMD = 0.1342 gr/cm²); B: sham (BMC = 0.3852 gr.; area= 3.852 cm²; BMD = 0.1279 gr/cm²); C: methylprednisolone acetate (BMC = 0.3582 gr.; area= 3.094 cm²; BMD = 0.1158 gr/cm²); D: methylprednisolone acetate + vitamin D3 (BMC = 0.3981 gr.; area= 3.014 cm²; BMD = 0.1321 gr/cm²). BMC, bone mineral content; BMD, bone mineral density.

(29) and osteocalcin (32) production by effecting osteoblast secretion. On the other hand, vitamin D metabolites has been accepted to increase intestinal calcium absorption (33) and osteoblast secretion (34) of osteocalcin and collagen I (35, 36), thereby, counteracting the effect of glucocorticoids.

A few controlled studies have been carried out to show these counteracting effects of methylprednisolone acetate and vitamin D3, however, their results have been contradicting. Tartrate resistance acid phosphatase (TRAP) is a lysosomal hydrolyser which has been shown to be released from spleen, prostate, erythrocyte and platelets. In serum, only in erythrocyte isoenzyme TRAP can be used as an index of osteoclast activity *i.e.* bones resorption (23). In the present study serum acid phosphatase activity was increased in methylprednisolone acetate group compared with groups A and B. It was decreased (not significantly) in group D which they were receiving vitamin D3. This lowering effect of vitamin D3 on acid phosphatase level may be resulted from short time treatment, as also shown in other studies. Bone mineral densitometry showed decreasing of bone mass in methylprednisolone acetate group and

increasing of that in vitamin D3 group which is also suggested by biochemical markers. Lips *et al.* (37) and Ooms *et al.* (38) have shown that daily supplementation with small doses of vitamin D2 or vitamin D3 can reduce the secondary hyperparathyroidism induced by vitamin D insufficiency and increase bone mineral density, but, there have been no prospective randomized controlled trial to evaluate the effect on vertebral osteoporosis and fracture rates on vertebrae.

In conclusion, the findings of present investigation indicate that using of vitamin D3 in methylprednisolone acetate induced osteoporotic rats could increase bone formation and decrease bone resorption. These finding were confirmed by biochemical markers and bone mineral densitometry.

Conflict of interests

The authors declare that they have no competing interests.

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