GLUCOCORTICOIDS INCREASE INFLAMMATION-MEDIATED OSTEOPENIA IN THE RAT

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The aim of the study was to assess if the administration of glucocorticoids, before the onset of inflammation-mediated osteopenia (IMO) induced by magnesium silicate in rats, influences IMO progress and if it has effects when administered simultaneously.

IMO in the rat is a method that leads to a fast bone mass decrease. It consists of injections s.c. in 8 points of the dorsal region, of $8 \times 400 \text{ mg}$ of talcum (magnesium silicate) sterile suspended in 0.5 ml saline solution.

The selected rats were of both sexes, with a body weight of 150 g, and were included in five study groups. All the animals received food daily, while the rats from only three groups had a daily dose of 0.25 mg/150g Prednisone in different periods of the experiment: before and/or at the same time with IMO. All the rats were measured and their weight was recorded before and after the treatment.

Calcium and magnesium values were measured from dry bone after sacrifice. Bone weight, volume and density, serum calcium and magnesium were also determined.

Bone calcium and magnesium contents were low in all the study groups versus the control group. Also, bone calcium and magnesium amounts were significantly reduced in the groups with IMO as compared to the animals that were only treated with Prednisone without IMO.

Results demonstrated that glucocorticoids administration before and at the same time with IMO increase bone calcium and magnesium deficiency.

Key words: glucocorticoids, osteopenia, rat, bone calcium and magnesium content.

INTRODUCTION

Glucocorticoids are known to cause osteoporosis (1). Both cortical and trabecular bone is lost, trabecular bone being more commonly affected (2). The incidence and severity of this kind of osteoporosis is a direct function of the dosage used and the length of therapy (2, 3).
Inflammation-mediated osteopenia (IMO) in the rat is a method that leads to bone mass decrease fast. Minne et al. have described this animal model for pathological loss of bone mass. It consists of injections s.c. in 8 points of the dorsal region, of 8 x 400 mg of talcum (magnesium silicate) sterile suspended in 0.5 ml saline solution (4-6). Over time, this method was used in Endocrinology Clinic of Cluj-Napoca for a lot of researches, about possibility of counteracting or reducing the rhythm of bone loss (4-7). Our study is a part of them.

The aim of the study was to assess if the administration of glucocorticoids before the onset of inflammation-mediated osteopenia (IMO) in the rat influences IMO progress and if it has effects when administered simultaneously with magnesium silicate.

**MATERIALS AND METHODS**

The selected rats were of both sexes, had a body weight of 150 ± 5 g each, and were included in five study groups. All the animals received food daily, while the rats from only three groups had a daily dose of 0.25 mg/150g body weight Prednisone in different periods of the experiment: before and/or at the same time with IMO. Prednisone was administered per os, mixed daily in the milk. All the rats were measured and their weight was recorded before and after the treatment. They were sacrificed after 21 or 42 days.

**Study groups**

1. Control group, with 10 animals. They were sacrificed after 42 days.
2. Inflammation-mediated osteopenia (IMO) group, with 10 rats with IMO, but without any treatment. They were sacrificed after 21 days.
3. IMOP group was formed of 10 rats with IMO and treated at the same time (21 days) with Prednisone.
4. IMOPP group included 10 rats treated before (21 days) with Prednisone, then IMO was induced and Prednisone therapy was continued for 21 days. The animals were sacrificed after 42 days.
5. P group: animals treated with Prednisone 42 days without IMO.

There were assessed serum calcium and magnesium, bone calcium and magnesium content by conventional atomic absorption spectrophotometry.

The left tibia was removed immediately after the animal’s death. Adjacent tissues were carefully removed from it. After that, the bones were left to dry 48 hours. The bones were weighed and their volumes were determined with a plethysmometer UGO-Basile. Bone density was calculated as report of mass/volume (g/cm³). Then, the bones were ashed in a muffle oven (600°C, 24 hours). The ash specimens were weighed and calcium and magnesium were determined.

**Statistical analysis**

Results were expressed as mean ± SD and they were compared using the Student’s paired t-test. Significance was determined for p<0.05.
RESULTS

There were compared the results obtained for each group of study with control group. For body weight, results are illustrated in Fig. 1.

![Figure 1](image1.png)

Figure 1. Comparison of body weight at the end of experiment for each group of study versus control.

Animals from groups with IMO had a body weight highly significant decreased versus the control group (p=0.001). P group had a mean body weight similar to control (p>0.05).

Decreased bone density was significantly high at IMO, IMOP and IMOPP groups (p<0.001) versus control. The P group, which was only treated with Prednisone, had a bone density similar to the control group (Fig. 2).

![Figure 2](image2.png)

Figure 2. Bone density at groups of study versus control.
All results for serum calcium were in normal limits. Mean serum calcium of IMO, IMOP and IMOPP groups, was significantly decreased comparative with control (p= 0.001). In the meantime, serum calcium of P group was decreased insignificantly as compared with control (p>0.05).

Results for serum magnesium were also in normal limits like for serum calcium. IMO group did not have a decrease of serum magnesium statistically significant versus control (p=0.066), but animals from groups which were treated with Prednisone had a significant decrease of magnesium (p=0.039 for IMOP; p<0.001 for IMOPP; p=0.003 for P).

For bone calcium content, results are compared in Fig. 3. Bone calcium content was significantly reduced at all four groups of study versus control (p<0.001).

![Figure 3. Bone calcium content at groups of study versus control.](image)

For bone magnesium content results were similar. Bone magnesium content was reduced statistically significant (p<0.001) for all the groups (Fig. 4).

![Figure 4. Bone magnesium content at groups of study versus control.](image)
A comparison between animals from groups with IMO versus the group which was only treated with Prednisone without IMO showed that bone density of rats from IMO, IMOP and IMOPP were significantly decreased versus rats from P group (p<0.001) (Fig. 5). There were also other differences of bone density between groups IMO-IMOPP (p=0.001) and IMOP-IMOPP (p<0.05).

Bone calcium content was decreased at groups with IMO as compared with P group (Fig. 6). There were also significant differences between groups IMOP-IMOPP and P-IMOPP (p<0.001).

For bone magnesium content the results are illustrated in Fig. 7. There was a significant decrease of bone magnesium content at IMO and IMOPP groups as compared with P group, while the comparison between IMOP and P groups was not statistically significant.
DISCUSSION

In humans, glucocorticoids cause a decrease in bone mass and an increase in fracture risk, particularly during the first few months of therapy (8). For this reason they are important risk factors for osteoporosis.

Glucocorticoids have direct negative effects on bone cells and calcium metabolism (8). They produce bone loss through many mechanisms such as reducing absorption of calcium from the gut, increasing urinary losses of calcium by decreasing its tubular reabsorption, and reducing the production of androgens in both the testis and the adrenals (9). They are also involved in decreasing calcitonin secretion, which is responsible for inhibiting osteoclastic production and activity. On the other hand, glucocorticoids induce a secondary hyperparathyroidism and an increased production of local resorption cytokines like OAF, interleukins and prostaglandins (10).

Glucocorticoids-induced osteoporosis is a low turnover type (11). At the beginning, glucocorticoids produce a decrease of osteoblast activity and proliferation, simultaneously with an increase of osteoclastic activity (12). Osteoblast and osteocyte apoptosis is increased in mice and humans receiving glucocorticoids (12). Over time, the decrease of osteoblasts leads indirectly to a reduction in pro-osteoclastic factors (e.g. RANKL), which caused an inhibition of osteoclastic proliferation and differentiation that can explain the fall of resorption that appears later (2, 13, 14).

There were tried some methods of inducing rapidly osteoporosis at animals such as mice, cats and rats. The most reliable model was rat. The osteopenia was produced in rats through calcium low diet, immobilization, orchidectomy or ovarectomy, but their most important criticism was a long time until osteopenia appeared (5, 6).
In 1984, Minne et al. have described a rat model of generalized osteopenia associated with chronic inflammation. The major advantage of this method is that it is rapidly followed by bone loss, which appears independent of PTH secretion or vitamin D metabolism. They also have found that indomethacin treatment inhibited the increase in serum calcium of the parathyroidectomized rat and blocked the inflammation-mediated loss of bone to extend (4).

Gozariu et al. demonstrated that ovariectomy associated with IMO has produced additional calcium and magnesium bone loss, hypercalcemia and a decrease of serum magnesium. They also found that calcitonin secretion was increased in young animals with IMO and serum levels of glucose and insulin were in normal range in IMO animals. They have reported that bone calcium and magnesium content after calcitonin, estrogens, anti-inflammatory drugs or anabolisant steroids administration was partially preserved (5).

In our study, body weight of animals with IMO decreased statistically significant, versus body weight of animals from the control group. The groups with IMO and Prednisone treatment had a significantly decreased body weight as compared to control group. The treatment with Prednisone only, without IMO, did not affect the body weight.

It has been demonstrated that IMO leads to trabecular bone loss (4-6). Bone density was significantly decreased at groups with IMO versus control (p<0.001), while P group has had a bone density similar to control group (p= ns). A longer period of therapy with Prednisone has induced an elevated decrease of bone density (IMOP vs IMOPP), confirming a negative effect of glucocorticoids on the IMO evolution.

According to others, in our study, serum calcium and magnesium were also in normal range (4-7), but there were statistical differences between study groups such as a significant decrease in serum calcium at IMO groups as compared with control and an increase of serum calcium at IMOPP group versus IMOP group. Increasing of serum calcium was correlated with decreasing in bone density at IMOPP group as a result of elevated bone resorption at this group of study similar to other evidence (4-6). Serum magnesium was in normal limits, but significantly lower at groups treated with Prednisone as compared to control, confirming again the negative effect of glucocorticoids on the IMO evolution. Bone calcium and magnesium contents were low in all the study groups versus the control group according with other studies (4-7).

**CONCLUSIONS**

Like other inflammatory process, IMO is a major factor of stress, which induced decreasing body weight and bone loss in rats. Treatment with Prednisone before and at the same time with IMO induced a greater decrease of bone density. Results demonstrated that glucocorticoids are one of the most important risk factors which can influence the evolution of experimental osteopenia.
Incontestably, our results confirmed the negative effect on bone loss caused by glucocorticoids administration in spontaneous evolution of inflammation mediated-osteopenia.

References