Original Article

Relation between the development of osteoporosis and osteonecrosis following glucocorticoid in a rabbit model

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Abstract

Background: There has been a recent increase in the number of patients suffering from bone and joint diseases, as a consequence of corticosteroids administration. There are more patients treated with low dose of GCs under long-term conditions in clinical, such as effect of GCs on Rheumatoid arthritis, Crohn's disease and Asthma patients. Hence, it was difficult for doctor to determine which problem occur first – OP or ON; however, there was no clinical report previously in the literature, and there was no effective animal model of OP and ON about low dose GCs. This study was conducted to develop rabbit models of glucocorticoid (GC)-induced femoral head ON and OP and to investigate the temporal relationship between the occurrence of the two events following administration of glucocorticoids.

Materials and Methods: Fifty six, 6 months old female rabbits were randomly divided into the GC group and control group (C). Rabbits received gluteal injections of methylprednisolone sodium succinate once a day for 4 weeks, while normal saline solution in the control group. Rabbits were sacrificed at 0, 2, 4, and 8 weeks. Hip magnetic resonance imaging was performed before the rabbits were sacrificed. Serum calcium (Ca), phosphorus (P), total cholesterol, and triglyceride levels were also measured. The bone mineral density (BMD) of femoral head and the femoral shaft were measured by dual-energy X-ray absorptiometry. The trabecular parameters of the femur and the 4th lumbar vertebrae (L4) were measured with a micro-computed tomography (μ-CT). Also, the femoral head was stained with hematoxylin-eosin staining.

Results: At 4 weeks in the GC group, the BMD of the femur reduced 33% and 22% in the femoral head and shaft; there was irregular intermediate to high T2-weighted images signals; μ-CT showed microfractures and cystic changes in the femoral head and L4 at 4 weeks. At 8 weeks in the GC group, the classical "line-like sign" indicating ON of the femoral head was observed in 64.3% of the rabbits.

Conclusion: A rabbit model of GC-induced OP and ON was developed by repetitive injection with small doses of GCs in the gluteal region. OP was observed at 4 weeks while ON developed at 8 weeks and followed a clear temporal pattern.

Key words: Rabbit model, glucocorticoids, osteonecrosis, osteoporosis
MeSH terms: Osteoporosis, osteonecrosis, glucocorticoid, rabbits

Introduction

There has been a recent increase in the number of patients suffering from bone and joint diseases, as a consequence of corticosteroids administration.[1-6] Managing these complex problems can be challenging, and thus an understanding of the disease process will improve patient management and disease prevention.

Various animal models of glucocorticoid (GC)-induced osteoporosis (OP) and osteonecrosis (ON) have been developed.[7-10] Combining GC with a lack of estrogen secretion has the most severe bone loss. Matsui's and Yamamoto's developed a GC-induced ON model by combining horse serum or endotoxin with high doses of GC.

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Other models using a single high-dose GC injection have also been widely reported.[11-13] However, the dose and application method of GC are not in accordance with the clinical treatment in a majority of cases. Most patients received long-term low dose steroids for their chronic medical conditions.

There are more patients treated with low dose of GCs under long-term conditions in clinical, such as effect of GCs on Rheumatoid arthritis, Crohn's disease and Asthma patients. Hence, it was difficult for doctor to determine which problem occur first – OP or ON; however, there was no clinical report previously in the literature, and there was no effective animal model of OP and ON about low dose GCs. Thus, a new model is necessary to understand better temporal relationship of GC-induced OP and ON.

This study aimed to develop a clinically relevant animal model of femoral OP and ON following administration of low-dose GC. We also investigated whether a temporal relationship exists between the development of OP, osteopenia and ON in the femoral head.

Materials and Methods

Animal

Rabbit care and experimental procedures were conducted with the approval of the Animal Care and Use Committee of our institute and were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Fifty six 6 months old (3.09 ± 0.15 kg body weight), female Japanese white rabbits were obtained and housed in the animal center of our institute. All the rabbits were skeletally matured according to the histological judgment and body weight. All rabbits were housed in a single pen, standard chow (0.8% calcium [Ca] and 0.5% phosphorus [P]) and tap water were free to eat and drink. The rabbits were then randomly divided into GC group (n = 24) and control (C) Group (n = 32).

Experimental animal model

After a 1-week quarantine and acclimatization period, all the rabbits were weighed to enable weight related dose adjustments, rabbits in the GC group received methylprednisolone sodium succinate (Pfizer Manufacturing Belgium NV, Belgium, 40 mg), which was injected into the right gluteus muscle at a dosage of 2 mg/kg/day (20 mg/ml) for 4 consecutive weeks. Rabbits in the C group were injected with 0.9% saline solution of the same quantity. Rabbits were weighed, and the doses were adjusted on a weekly basis. At the end of the experiment, 0, 8, 8, and 8 rabbits in the GC group and 8, 8, 8, and 8 rabbits in the C group were killed with a lethal dose of pentobarbital sodium at 0, 2, 4, and 8 weeks, respectively.

Serum samples

Blood samples were taken at 0, 2, 4, and 8 weeks following an overnight fast. The samples were obtained at the same time between 9:00 and 10:00 AM and stored in ice-cooled water until centrifugation and aliquotting. Ca, P, total cholesterol (TC), and triglyceride (TG) levels were measured with a fully automated technique (Roche Hotline was E900, USA).

Magnetic resonance imaging

The "line-like sign" was considered a sign of the necrosis of the femoral head in the magnetic resonance imaging (MRI). MRI (Germany Siemens Magneton Vision 3.0T) images were obtained to examine both hip joints before the rabbits were sacrificed. Briefly, rabbits were anesthetized by inhalation of halothane and were placed supine in an imaging coil with the hips and knees flexed at right angles. Four contiguous multi-section images in the coronal plane were obtained. The imaging parameters of the 3.0T Trio total imaging matrix were a 2.2 mm slice thickness, a 307 × 384 imaging matrix, a field 140 mm of view. T1-weighted (T1-W, repetition time [TR]/echo time [TE] 650/22 ms), T2-weighted (T2W, TR/TE 3000/36 ms), and fat suppression T1-weighted images (T1WI) were obtained with a spin echo sequence.

Microcomputed tomography, bone mineral density, and histomorphometry

Following animal sacrifice, microcomputed tomography (μ-CT) (Laboratory for Optoelectronics, Huazhong University, China) was used to quantify structural parameters of the lumbar vertebrae (L4) and the femoral head. Trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation, bone surface/bone volume, and bone volume/total volume (BV/TV) were determined. For the free rabbit femur specimens, the bone mineral density (BMD) in the femoral shaft (middle third of the femur) and femoral head (circular field of the femoral head) was measured with dual-energy X-ray absorptiometry (Hologic 2000 Plus type, USA) as previously described;[14] OP is universally defined as a decrease in BMD by >2.5 standard deviation below the mean. 1-mm diameter collimator on the X-ray output and specific software for small animals were used (version 6.2). A region of interest of the middle third of the femur and the femoral head was defined for the cortical bone and the femoral head BMD values.

The femoral head was sectioned in the coronal plane to analyze empty lacuna as previously described.[15] The femoral head was fixed in buffered 4% (w/w) paraformaldehyde saline (pH 7.4) at 4°C and decalcified in ethylene diamine tetraacetic acid (pH 7.4) at 37°C. They were then cut along the coronal plane to visualize trabecular bone and

bone marrow. The specimens were embedded in paraffin, cut into 4-mm slices and stained with hematoxylin and eosin (H and E). Evidence of trabecular bone necrosis was defined as the presence of lacunae entire empty of osteocytes, decreased number of subchondral vessels, and increase in size of fat cells, as measured by micrometer under a light microscope; the size of the red blood cell was set as reference, at ×100 magnification. Fifty osseous lacunae for each high power field were selected to calculate a percentage.

Statistical analyses

Data were presented as mean ± standard error of the mean. The analyses were carried out with SPSS 18 (SPSS Inc., Chicago, IL, USA). For the normally distributed data, the ANOVA was used to determine differences; for the repeated measurements data, the ANOVA was used to determine differences. $P = 0.05$ was considered statistically significant.

Results

Body weight

Both groups had a significant decrease in body weight during the injection period, but there was no significant difference between the two groups. The loss of body weight was more obvious during the second week of injection than during the first 2 weeks of injection ($P < 0.05$) [Figure 1].

Serum biochemical parameters

There was no change in the concentrations of serum Ca, P, TC and TG in the C group. The levels of serum Ca and P were markedly elevated in the GC group at 2 weeks ($P < 0.05$), then began to decline after 2 weeks, and returned to baseline levels at 8 weeks. The TC and TG levels increased in the GC group and there were statistically significnt differentces at 2 and 4 weeks when compared to the control group ($P < 0.01$). However, the TC and TG levels returned to baseline levels by 8 weeks [Figure 2].

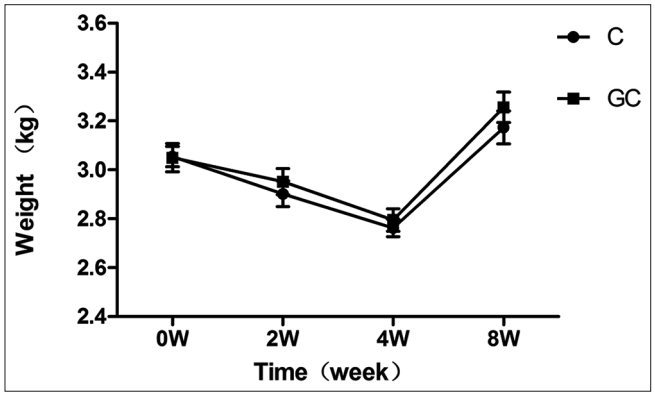

Figure 1: Weight monitoring: Both groups had a significant decrease in body weight, but there was no significant difference between the two groups. The loss of body weight was more obvious during the second weeks than the first 2 weeks ($P < 0.05$), there was no significant difference when compared to the control group ($P > 0.05$)

Bone mineral density measurements

In the C group, the BMD of cancellous bone and cortical bone in the femoral head and femoral shaft increased over 8 weeks, but there was no significant difference between each time point ($P = 0.908$ and $P = 0.844$). In the GC group, BMD decreased in the femoral head and femoral shaft at 2 weeks and there was statistically significant difference when compared to the C group ($P < 0.05$). The BMD in the GC group decreased in the femoral head and femoral shaft after GC intervention, but the BMD go back to normal, increasing substantially after the injections were stopped after 4 weeks. However, there was still a significant difference between two groups at 8 weeks in the femoral head ($P < 0.05$) and in the femoral shaft ($P < 0.01$) [Figure 3].

Microcomputed tomography

As shown in Table 1, in the C group, μ-CT indicated that the Tb.N of the femur and L4 increased gradually throughout the study. Also, the trabecular bone structure in the C group was uniform and continuous [Figure 4]. In the GC group, the trabecular bone structure was disordered and uneven and the trabecular bone collapsed into fractures. Also, there were subchondral cystic changes in the femoral head, and the number and size of the lesion gradually increased at 4 and 8 weeks, respectively [Figure 4]. The Tb.N, Tb.Th, and BV/TV of the femoral head and L4 in the GC group were significantly decreased when compared with the C group at 4 weeks ($P < 0.01$), while only Tb.N and BV/TV in the femoral head and BV/TV in L4 were significantly decreased at 8 weeks ($P < 0.05$).

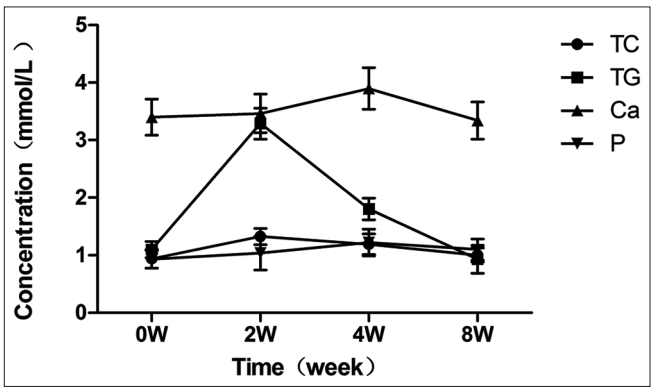

Figure 2: Biochemical measurements: In the glucocorticoid group, there was no change in the concentrations of serum calcium, phosphorus, total cholesterol, and triglyceride in the control group. The levels of serum calcium and phosphorus were markedly elevated at 2 weeks ($P < 0.05$), the total cholesterol and triglyceride levels increased and there were statistically significant differences at 2 and 4 weeks compared to the control group ($P < 0.01$). However, the calcium, phosphorus, total cholesterol, and triglyceride levels returned to baseline levels by 8 weeks

Magnetic resonance imaging

There was a homogeneous low signal on the T1-WI in the C group. In the GC group, the T2-weighted images (T2-WI) showed minor changes at 2 weeks and were not suggestive of avascular necrosis. At 4 weeks, necrosis was observed in 18.7% (3 femoral heads of 8 rabbits) of the cases [Figure 5]. Furthermore, necrosis was apparent in 64.3% (9 femoral heads of 7 rabbits) of the cases at 8 weeks in the GC group [Table 2].

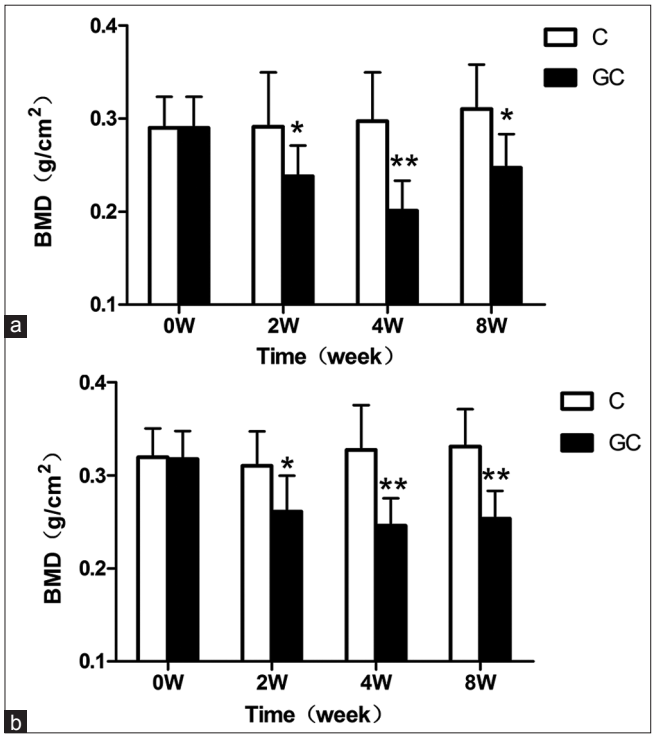

Figure 3: A bar diagram (a and b) showing bone mineral density in the femoral head and shaft. In the glucocorticoid group, bone mineral density decreased in the femoral head (a) and femoral shaft (b) at 2 weeks compared to the C group (*$P < 0.05$). The bone mineral density began to increase substantially after the injections were stopped after 4 weeks (**$P < 0.01$); however, there were still significant differences between two groups in the femoral head (*$P < 0.05$) and in the femoral shaft (**$P < 0.01$)

Histological examination

In the C group, H and E staining of the femoral head showed uniform and continuous trabecular bone structure, which was similar to the μ-CT results. In the GC group, at 2 weeks, there were greater number of fat cells as compared to time 0 or to the C group, and the empty lacunae rate increased to 29.5%. At 4 weeks [Figure 6], the trabecular bone structure was disorderly and uneven, the trabecular bone collapsed into fractures, there were subchondral cystic changes, a sclerosis zone in the femoral head was observed, the percentage volume of the marrow cavity occupied by adipose tissue increased, and a large number of fatty cells and a small amount of hemopoietic tissue were observed in the marrow cavity. Also, the percentage of empty lacunae increased significantly to 35.7% in the trabecula. Repairing necrotic bone with granulation tissue was not observed at the border of the sclerosis zone. At the end of 8 weeks, the cystic changes and sclerosis zone did not significantly increase. However, the number of osteoblasts at the trabecular surface increased when compared to 4 weeks. Also, the number of empty lacuna decreased gradually to 32.3%, and the amount of new trabecular bone increased.

Discussion

Animal models of GC-induced OP and ON have been reported previously. Of the documented models, GC

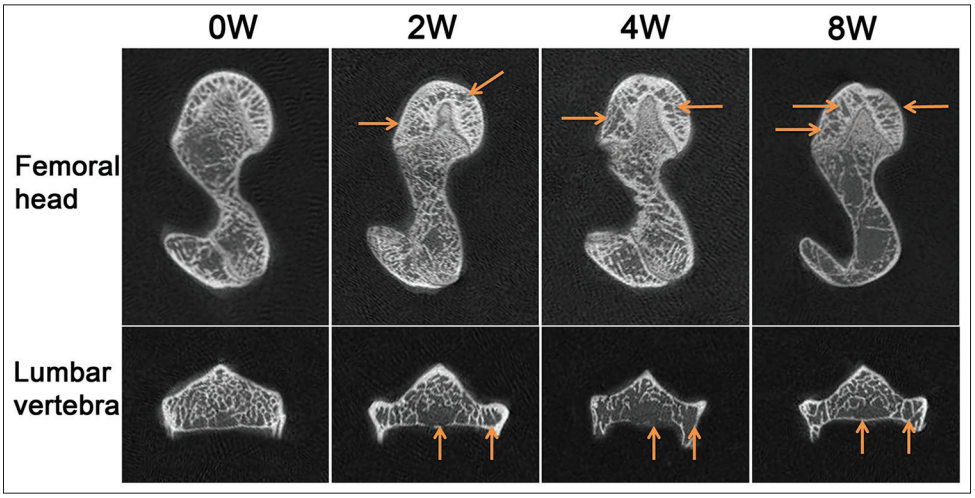

Figure 4: Micro-computed tomography image of the femoral head and lumbar region showing the peculiarity of uniformity and solid reticulation at 0 weeks. There were subchondral cystic changes in the femoral head after 2 weeks, the number and size of these lesions increased at 4 and 8 weeks. The trabecular bone structure in the lumbar vertebrae L4 was uniformity and solid reticulation at 0 week, then disordered and uneven after 2 weeks, and the trabecular bone collapsed into fractures after 4, 8 weeks

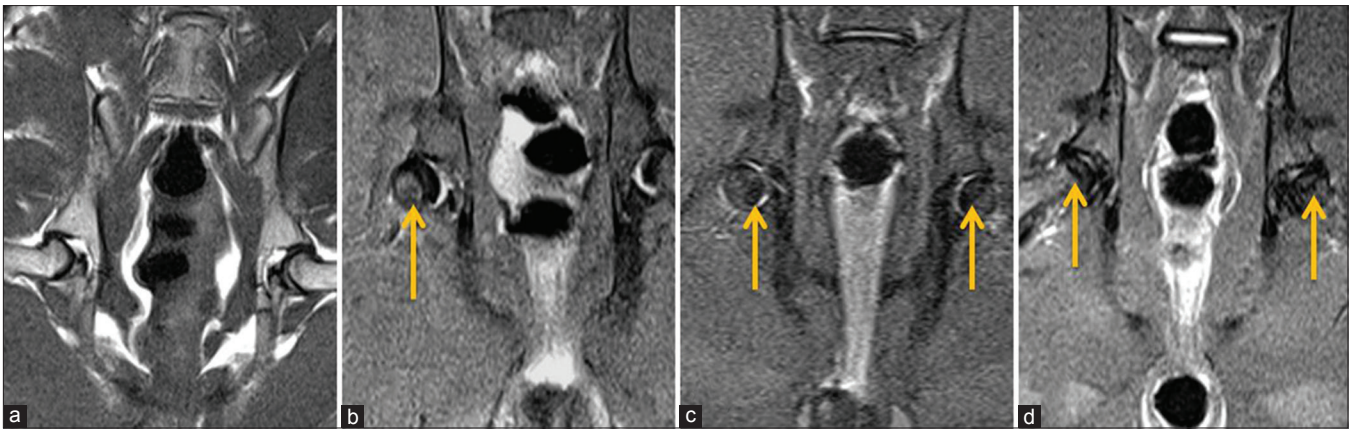

Figure 5: Magnetic resonance imaging of the femoral head. The femoral head of the C group was homogeneous low signal on the T1-weighted images at 0 week (a). The head of the glucocorticoid group demonstrated irregular intermediate to high T2-weighted images signals at 2 weeks (b), 4 weeks (c), 8 weeks (d), "line-like sign" – a high signal intensity area surrounded by low signal intensity areas were observed after 8 weeks. The arrow shows localized necrotic area

Table 1: μ-CT analysis of the femoral head and lumbar vertebra in the GC group

Classify	Group	Femoral head				Lumbar vertebra			
		0 week	2 weeks	4 weeks	8 weeks	0 weeks	2 weeks	4 weeks	8 weeks
Tb.N (N)	C	3.93±0.36	3.97±0.37	4.13±0.34	4.12±0.37	2.97±0.32	2.97±0.31	3.05±0.34	3.05±0.33
	GC		3.56±0.33[a]	3.42±0.25[b]	3.83±0.28[a]		2.72±0.28[a]	2.57±0.25[b]	2.83±0.31
Tb.Sp (μm)	C	316±30	314±31	316±35	316±33	346±38	349±34	339±38	344±35
	GC		349±32	360±29[a]	333±30		402±36[a]	421±30[b]	359±36
Tb.Th (μm)	C	240±26	245±25	241±28	238±24	198±21	198±25	204±21	202±27
	GC		192±25[a]	187±28[b]	234±25		147±21[b]	148±19[b]	182±19
BS/BV (%)	C	10.4±1.2	10.0±1.3	10.2±1.4	10.4±1.2	8.9±1.2	8.8±1.2	8.9±1.2	8.9±1.3
	GC		11.7±1.1[a]	11.9±1.3[a]	10.9±1.1		10.4±1.3[a]	10.8±1.2[a]	9.4±1.1
BV/TV (%)	C	38±4.6	40±4.4	43±4.2	42±4.5	36±3.8	38±4.0	37±3.6	38±3.9
	GC		33±3.1[a]	27±2.8[b]	36±3.2[a]		32±3.1[a]	27±2.3[b]	34±3.6[a]

Each time group was compared to 0 weeks [a]$P<0.05$, [b]$P<0.01$. Tb.N=Trabecular number, Tb.Sp=Trabecular separation, Tb.Th=Trabecular thickness, BS/BV=Bone surface/bone volume, BV/TV=Bone volume/total volume

Table 2: Femoral head results in the GC group

Time (weeks)	Rabbit number (*n*)	Necrosis number (leg)	Necrosis rate (%)
0	4	0	0
2	8	0	0
4	8	3	18.7
8	7	9	64.3

combined with a lack of estrogen secretion has been shown to be the most effective.[7-10] GC-induced ON models often combine horse serum or endotoxin with high doses of GC.[11-13] However, the dose and application method of GC in these models are not in accordance with clinical treatments because most patients with chronic medical conditions are treated with a low dose of GC. Thus, it is necessary to establish a model of GC-induced OP and ON using low doses of GC. It is also important to better understand the temporal relationship between the development of OP, femoral head osteopenia, and ON.

Rabbits have been widely used for bone disease models because they have early skeletal maturity, high bone metabolic activity, and long-term stability.[1] We chose female rabbits in this study because more women suffer from OP in the clinical setting. Our model had a low mortality, which is relatively high in single, high-dose GC models or combined models. In a previous study, Miyanishi *et al.*[16] showed that the administration of methylprednisolone acetate resulted in more side effects when compared to other GC drugs. Also, Castañeda *et al.* and Eberhardt *et al.*[1,17] demonstrated that methylprednisolone acetate at 1.7 mg/kg/day for 4 weeks could induce OP and femoral head osteopenia or osteocyte death. In this study, we chose to treat rabbits with methylprednisolone acetate at 2 mg/kg/day for 4 weeks.

Although there was a low mortality rate (only one rabbit died) in our study, there was a significant decrease in body weight during the injection period in both groups. This is consistent with some previous reports.[7,18] Other reports in sheep suggest that the body weight remained unchanged or increased after administration of GC.[19,20] We believe that the main reason for body weight loss in rabbits was the pain secondary to gluteus injection that was given

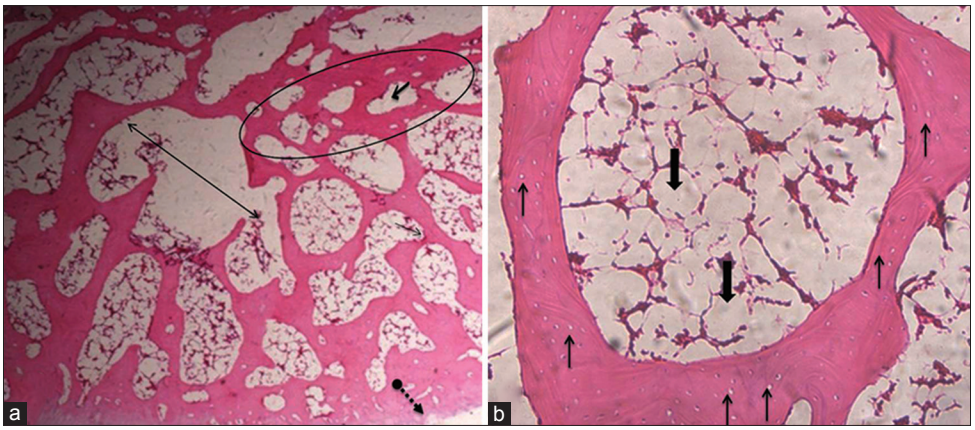

Figure 6: Histomorphology of the femoral head at 4 weeks. It is mainly composed of osteoporosis (a) double-headed arrow: Bone cyst; dashed arrows: Thinning subchondral bone; oval: Hardened zone; at oblique arrow: New bone area. The magnification is ×40. There is large number of fatty cells and empty lacunae (b) (thin arrow: Empty lacunae; and thick arrow: Fat accumulation. The magnification is ×200)

every day. This may have caused rabbits to eat less and reduce their activity, especially in the last 2 weeks of the experiment.

Serum biochemical values were used to determine whether methylprednisolone acetate was effective and whether there were anabolic or catabolic changes in the skeletal system post treatment. In the GC group, the Ca and P levels were markedly elevated at 2 weeks, suggesting that bone catabolism was higher than anabolism. This has been observed in previous studies.[12,16] Between 2 and 4 weeks, the Ca and P levels began to decline, which is inconsistent with a report by Kabata *et al*.[21]

In this study, bone loss occurred more in the cancellous bone region, with maximum bone loss in the femoral head (33%) and shaft (22%) at 4 weeks. The change in bone loss was small at 8 weeks, but there was still a statistical significance when compared with the C group ($P < 0.05$). Recent studies by Baofeng *et al*.[7] have shown that methylprednisolone acetate (1 mg/kg/day) treatment for 4 weeks can induce as much as 10% bone loss in the lumbar region in rabbits and by as much as 22% at 8 weeks. Castañeda *et al*.[1] treated rabbits with a higher methylprednisolone acetate dose (1.5 mg/kg/day) for 4 weeks to induce 17.4% bone loss in the lumbar spine. Based on these studies, we hypothesized that there was a correlation between the degree of OP and the GC dose.

MRI is sensitive for the detection of early ON of the femoral head and has become the standard for detecting early ON of the hip in clinical settings. In this study, there was a region of high signal intensity on T2WI with a necrosis rate 18.7% at 4 weeks, which increased to 64.3% at 8 weeks. Chen *et al*.[15] suggested that MRI lacks sensitivity to diagnose early ON (ARCO 0) if there is no evidence of necrosis following osteogenic repair. They also suggested that initial MRI signal variations could be observed at 4 weeks while the "line-like sign" is primarily observed only at 16 weeks.

In our study, at 4 weeks, H and E staining and μ-CT showed that the trabecular bone structure was disorderly and uneven and that the bone trabecula collapsed into fractures. In addition, there were subchondral cystic changes, a hardening zone in the femoral head, the percentage volume of the marrow cavity occupied by adipose tissue increased and the percentage of empty lacunae increased significantly to 35.7% in the trabecula. These findings were subtle or not observed by MRI, but were easily identified with H and E staining. At 8 weeks, H and E staining was completely consistent with MRI in the femoral head. Several researchers[12,13,15,20,22] believe that necrotic lesions induced by single high-dose GC occur in the proximal femur metaphysis, which is different from that of humans where it is confined only to the femoral head. In our study, H and E staining, μ-CT and MRI indicated that necrosis occurred in the femoral head. Unfortunately, the femoral head did not collapse in any of the rabbits in our study. One possible reason could be that rabbits have relatively smaller hind legs and bear less weight as four-legged animals compare to two-legged human beings. Bowers *et al*.[23] suggested that femoral head collapse occurs due to a decrease in trabecular mechanical function. Motomura *et al*.[24] suggested that femoral head collapse begins the boundary of large necrosis lesions. However, this study was largely inconclusive and requires further investigation.

In this model, there was more bone loss in the femoral head and wider cystic changes when compared to the femoral head. Fessel *et al*.[25] found that there was a greater degree of OP in patients with femoral head necrosis. Tan *et al*.[26] also found that there was a relationship between GC-induced OP and GC-induced ON. They showed that GC leads to OP by suppressing bone marrow mesenchymal

stem cell proliferation and differentiation and strengthening osteoclast activity.

The exact mechanisms of GC-induced OP and ON have not been clearly elucidated. GC decreases bone formation and increases bone resorption, leading to bone loss. More specifically, GC directly inhibits osteoblasts from producing new bone and decreases osteoblast proliferation, while increasing osteoclast activity.[27] Also, the GC receptor also plays an important role in the development of bone loss.[28,29] Several other mechanisms have been implicated in the pathogenesis of femoral head ON, including intraosseous hypertension, intravascular fat emboli, coagulation, and compression of vessels by progressive accumulation of marrow fat stores.[16,20,21,30] Also, GC causes avascular necrosis via an apoptotic mechanism of osteocytes and osteoblasts.[31] Once ON occurs, GC also inhibits bone regeneration.[32] Furthermore, Wang *et al*. demonstrated that neural lesions may induce ON following GC administration.[30]

There is a significant correlation between the pathogenesis of GC-induced femoral head ON and OP. In our study, GC induces terminal adipocyte differentiation and hyperlipidemia at 2 weeks and then fat embolism and intraosseous fat embolism and intraosseous hypertension were found to be present in the histologic at 4 weeks, compressed vessels and decreased bone blood supply in the femoral head, it finally leads to apoptotic of osteocytes, osteoblasts, and endothelial cell, but only bone loss in femur and vertebral L4 were observed at 4 weeks. In another way, GC directly suppressed bone formation due to decreased activity and shortened lifespan of osteoblasts, and also promoted of osteoclasts survival and strengthen osteoclast activity, which finally cause Ca and phosphate loss at 2 weeks, the empty lacunae rate in the femoral head increased to 29.5% at 2 weeks. At 4 weeks, the trabecular bone structure was disorder and uneven with μ-CT and H and E staining, there were subchondral cystic changes, a sclerosis zone in the femoral head was observed, the percentage volume of the marrow cavity occupied by adipose tissue increased, and the percentage of empty lacunae increased significantly to 35.7% in the trabecular. At 8 weeks, these findings were obvious and observed by MRI, and were easily identified with H and E staining, and H and E staining was completely consistent with MRI in femoral head. And, finally disrupted the osteocyte network of the femoral head, but the femoral head did not collapse in any of the rabbits in our study.

The limitations of this study are: First, although maximum bone loss was 33% and 22% in the femoral head and femoral shaft, respectively, and necrotic lesions were mainly located in the femoral head, accurate titration of the dosage and mode of administration of GC to accurately mimic the condition leading to a human femoral head ON model could not be accurately defined. We also believe that while this study provides valuable insight into the temporal relationship of the two, further investigation of the readability, stability and reproducibility of this rabbit model is needed.

A rabbit model of OP and femoral head ON was developed by repetitive injections with small doses of GC in the gluteal region, this model represents long-term GC treatment, and this situation resembles actual clinical situation, but Low dose GC causes ON were relatively rare in clinical practice. An extended effect was reported after cessation of GC that maintained osteopenia bone for more time, further studies, including molecular, bone biomechanics, onset of an ischemic event, dynamic histomorphometry, and *in vitro* investigations, are needed to characterize further this animal model. Whereas some alternatives have been partially characterized, further studies seem warranted to advance the use of other candidate animal models or to explore potential variations of existing models, and different doses or formulations might work better. If there are data about negative results in other animals, more works are required to repeat to determine the relationship between the OP and the ON.

Conclusion

A rabbit model of OP and femoral head ON was developed by repetitive injections with small doses of GC in the gluteal region. OP was observed at 4 weeks while ON developed at 8 weeks and followed a clear temporal pattern.

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Conflicts of interest

The authors have declared that no competing interest exists.

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