Dose Effects of Corticosteroids on the Development of Osteonecrosis in Rabbits

GORO MOTOMURA, TAKUAKI YAMAMOTO, TAKAHIKO IRISA, KEITA MIYANISHI, KENJIRO NISHIDA, and YUKIHIDE IWAMOTO

ABSTRACT. ***Objective.*** The relationship between dose of corticosteroids and the prevalence of osteonecrosis (ON) has not been established. We examined the dose effects of corticosteroids on the development of ON in a rabbit model.

Methods. Rabbits were injected once intramuscularly with 1 (12 rabbits), 5 (12 rabbits), 20 (20 rabbits), and 40 (25 rabbits) mg/kg of methylprednisolone acetate (MPSL) into the right gluteus medius muscle. Four weeks after the MPSL injection, the proximal and distal parts of both the femora and humeri were histopathologically examined for the presence of ON. Hematological examinations were performed before and after the corticosteroid injection.

Results. In rabbits with 1, 5, 20, and 40 mg/kg MPSL, the incidence of ON was 0, 42%, 70%, and 96%, respectively. The dose of MPSL showed a significant association with the incidence of ON. Histologically, reparative tissues around the ON sites were observed in the rabbits with 5 mg/kg MPSL, but not observed in rabbits with 20 and 40 mg/kg MPSL. On hematological examination, hyperlipidemia and thrombocytopenia were most apparent in the rabbits receiving 40 mg/kg MPSL.

Conclusion. The study suggested that the dose of corticosteroids plays an important role in the development of ON in rabbits. The repair process was also found to be influenced by the dose of corticosteroids. Corticosteroid-induced hyperlipidemia and thrombocytopenia seemed to be associated with the incidence of ON. (First Release Oct 1 2008; J Rheumatol 2008;35:2395–9; doi:10.3899/jrheum.080324)

Key Indexing Terms:
OSTEONECROSIS CORTICOSTEROID DOSE ANIMAL MODEL

Osteonecrosis (ON) of the femoral head is often observed in young patients receiving corticosteroids for diseases such as systemic lupus erythematosus (SLE) and renal transplantation[1,2]. Once collapse of the femoral head occurs, hip joint destruction gradually progresses, and total hip arthroplasty is generally needed.

High-dose corticosteroid treatment has been considered to be one of the risk factors for the development of ON[3-6]. A recent prospective study showed that 13 of 15 patients with ON (87%) had a history of corticosteroid pulse therapy with 1000 mg/day of methylprednisolone for treatment of SLE[3]. On the other hand, some studies showed no differences in the dose of corticosteroids between patients with ON and those without ON[7-9]. To date, the association between dose of corticosteroids and development of ON has not been established for all conditions.

When rabbits are treated with a single injection of high-dose methylprednisolone acetate (MPSL, 20 mg/kg), which corresponds to corticosteroid pulse therapy in the human, about 70% develop ON in the metaphysis or diaphysis (but not in the epiphysis) reproducibly[10-12]. Using this model, we examined the dose effects of corticosteroid on the development of ON in rabbits.

MATERIALS AND METHODS

We utilized a rabbit model of corticosteroid-induced ON in this study[10]. All experiments were conducted in accord with the Guidelines for Animal Experiments of Kyushu University, the respective government law and notification, and the Committee on Ethics in Japan.

Animals. Sixty-nine adult (defined as animals with closed growth plates) male Japanese white rabbits (Kyudo, Tosu, Japan) were housed at the Animal Center of Kyushu University and were maintained on a standard laboratory diet and water. Rabbits ranged in age from 28 to 32 weeks.

Treatment. The rabbits were injected once intramuscularly with 1 (12 rabbits), 5 (12 rabbits), 20 (20 rabbits), and 40 (25 rabbits) mg/kg of MPSL into the right gluteus medius muscle. Four weeks after the MPSL injection, the rabbits were sacrificed and tissue specimens were prepared as described[10].

Evaluation of ON. Whole areas of the proximal one-third and distal condyles of both the femora and humeri (totaling 8 regions) were examined histopathologically for the presence of ON. A diagnosis of ON was made by a blinded examination by 3 independent investigators (GM, TY, KM). A

From the Department of Orthopaedic Surgery, Kyushu University, Fukuoka, Japan.

Supported in part by a Grant-in-Aid in Scientific Research (No. 18591665) from Japan Society for the Promotion of Science and Research Grant for Intractable Diseases from the Ministry of Health and Welfare of Japan.

G.M. Motomura, MD, PhD; T.Y. Yamamoto, MD, PhD; T.I. Irisa, MD, PhD; K.M. Miyanishi, MD, PhD; K.N. Nishida, MD, PhD; Y.I. Iwamoto, MD, PhD, Department of Orthopaedic Surgery, Kyushu University.

Address reprint requests to Dr. T. Yamamoto, Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
E-mail: yamataku@ortho.med.kyushu-u.ac.jp

Accepted for publication July 4, 2008.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

positive diagnosis was determined based on the diffuse presence of empty lacunae or pyknotic nuclei of osteocytes within the bone trabeculae, accompanied by surrounding bone marrow cell necrosis[10]. If the diagnoses differed between the 3 investigators, a consensus was reached by discussion of the histological findings without the knowledge of the sample group. The rabbits possessing at least one osteonecrotic lesion in the 8 areas examined were considered to be rabbits with ON.

Hematological examination. Blood samples were obtained from fasting rabbits prior to experimentation (0 week) and 1, 2, 3, and 4 weeks after the corticosteroid injection. We examined the blood levels of cholesterol, triglycerides, and platelets.

Statistical analysis. The number of ON-positive rabbits was compared using Fisher's exact probability test. The relationship between the dose of MPSL and prevalence of ON was analyzed by chi-square test. Hematologic data obtained at each timepoint were analyzed by one-factor analysis of variance with Scheffe's post hoc test. The statistical analyses were performed using the Stat View J-5.0 program (SAS Institute, Cary, NC, USA). P values less than 0.05 were considered to be significant.

RESULTS

One rabbit receiving 40 mg/kg MPSL died of pneumonia at 3 weeks after the injection. After the exclusion of this rabbit, 68 rabbits were evaluated.

Macroscopic and histopathological features of ON. ON in both the femur and humerus was observed macroscopically as yellowish-colored areas in the metaphysis and diaphysis. Histologically, ON lesions demonstrate an accumulation of bone marrow cell debris and bone trabeculae possess empty

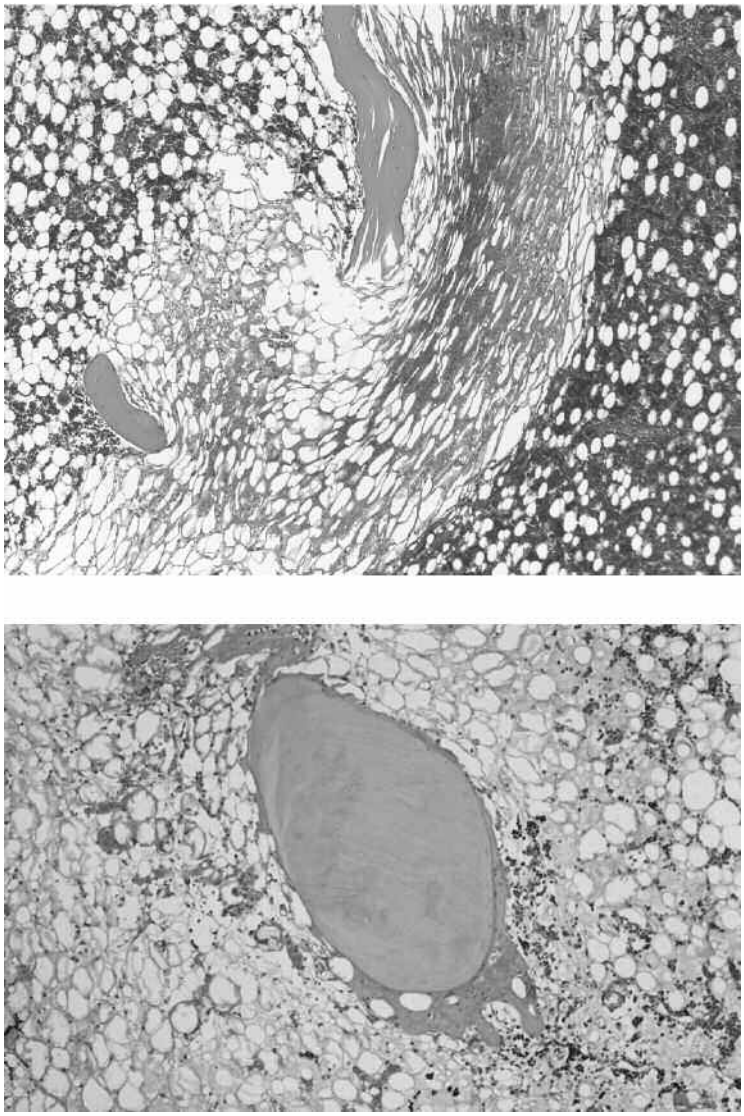

Figure 1. Histopathological features of osteonecrosis 4 weeks after corticosteroid injection in rabbits. Bone marrow tissues contain necrotic cell debris and trabeculae possess empty lacunae. A. In rabbits receiving 40 mg/kg MPSL, little reparative tissue is observed. B. In rabbits with 5 mg/kg MPSL, ON lesions are accompanied by evidence of reparative process, including aggregation of macrophages and fibrous tissue, and small amount of appositional bone formation. (H&E stain; original magnification, A ×40, B ×100.)

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

lacunae. These findings were consistent for all osteonecrotic tissues. Little reparative response was observed in rabbits receiving 20 mg/kg and 40 mg/kg MPSL (Figure 1A). On the other hand, in rabbits with 5 mg/kg MPSL, ON lesions were accompanied by an adjacent reparative process, including aggregation of macrophages and fibrous tissue invasion, and small amount of appositional bone formation (Figure 1B). In the rabbits with 1 mg/kg MPSL, ON was not observed.

Prevalence of ON. In the rabbits with 5, 20, and 40 mg/kg MPSL, the incidence of ON was 5/12 (42%), 14/20 (70%), and 23/24 (96%), respectively. The incidence rate of ON in the rabbits with 40 mg/kg MPSL was significantly higher than the rates observed in rabbits with 5 and 20 mg/kg ($p < 0.001$ and $p < 0.05$, respectively; Figure 2). In addition, the dose of MPSL showed a significant association with the incidence of ON ($p = 0.0016$). Multifocal ON lesions were identified in all groups. The mean ± SD number of lesions was 1.2 ± 0.4 in rabbits with 5 mg/kg, 1.3 ± 0.5 in rabbits with 20 mg/kg, and 2.4 ± 1.2 in rabbits with 40 mg/kg.

Hematological examination (Figure 3). The serum cholesterol levels in rabbits receiving 40 mg/kg MPSL were significantly higher than those observed in rabbits with 20 mg/kg MPSL ($p < 0.0001$ at 3 weeks; $p < 0.01$ at 4 weeks), rabbits with 5 mg/kg MPSL ($p < 0.005$ at 2 and 3 weeks; $p < 0.05$ at 4 weeks), and rabbits with 1 mg/kg MPSL ($p < 0.0001$ at 2, 3, and 4 weeks). The serum cholesterol levels in rabbits with 20 mg/kg MPSL were significantly higher than those in rabbits with 1 mg/kg MPSL ($p < 0.01$ at 2 weeks).

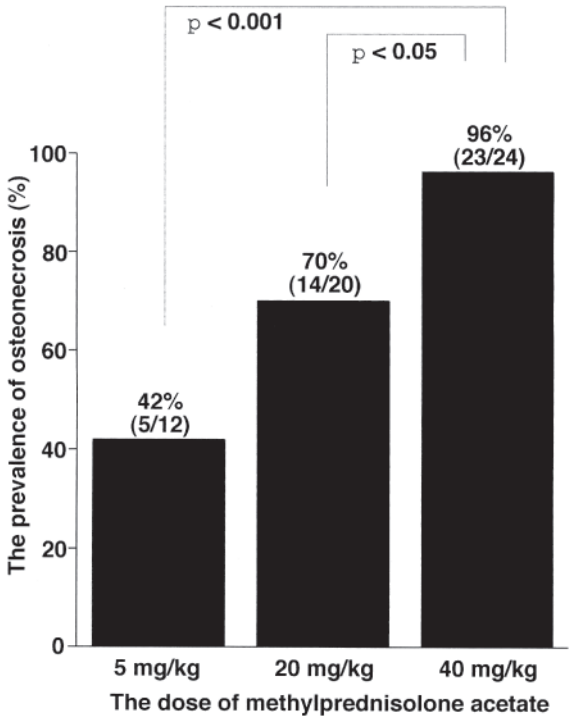

Figure 2. Prevalence of ON in each study group. The incidence of ON in rabbits receiving 40 mg/kg MPSL (96%) was significantly higher than rates observed in rabbits with 5 mg/kg (42%) and 20 mg/kg (70%) ($p < 0.001$ and $p < 0.05$, respectively).

Serum triglyceride levels in rabbits with 40 mg/kg MPSL were significantly higher than those in rabbits with 20 mg/kg MPSL ($p < 0.01$ at 3 weeks), rabbits with 5 mg/kg MPSL ($p < 0.001$ at 3 weeks; $p < 0.005$ at 4 weeks), and rabbits with 1 mg/kg MPSL ($p < 0.05$ at 2 weeks; $p < 0.0005$ at 3 weeks; $p < 0.005$ at 4 weeks).

Platelet levels in rabbits with 40 mg/kg MPSL were significantly lower than those in rabbits with 20 mg/kg MPSL ($p < 0.0001$ at 4 weeks), rabbits with 5 mg/kg MPSL ($p < 0.05$ at 2, 3, and 4 weeks), and 1 mg/kg MPSL ($p < 0.05$ at 1 week; $p < 0.0001$ at 2 weeks; $p < 0.0005$ at 3 weeks). The platelet levels in rabbits with 20 mg/kg MPSL were significantly lower than those in rabbits with 5 mg/kg MPSL ($p < 0.005$ at 2 weeks) and rabbits with 1 mg/kg MPSL ($p < 0.0001$ at 2 weeks; $p < 0.005$ at 3 weeks).

DISCUSSION

In our study, the prevalence of ON in rabbits increased in association with the increased dose of MPSL, suggesting the dose-dependent effect of MPSL on development of ON. In addition, the excessive corticosteroid treatment (40 mg/kg MPSL) resulted in a very high prevalence of ON (96%). These findings strongly suggest that the dose of corticosteroids may play an important role in the development of ON in rabbits.

In humans, several studies have suggested a possible relationship between the dose of corticosteroid and prevalence of ON. In renal transplant patients, the prevalence of ON clearly decreased from 20% to 4% after introduction of cyclosporine and the subsequent reduction of the corticosteroid dose[13]. However, there also have been conflicting reports regarding the dose of corticosteroid and prevalence of ON. In patients with spinal cord injury, no cases of ON were found after short-term megadose MPSL[14], suggesting that the dose of corticosteroids is not the only factor involved in development of ON. Many of the underlying conditions for which corticosteroids were administered, such as vasculitis, may also play an important role in development of ON[15]. In addition to the complicated etiology of ON, we should also take the variability of prescription combinations of corticosteroids, such as the duration and the route of administration, into consideration for assessing the dose effects of corticosteroids in humans.

The current data suggest that significant differences in the changes of serum lipids and platelet levels during the period from 1 to 2 weeks after corticosteroid injection were associated with the prevalence of ON. In rabbits with 40 and 20 mg/kg injections of MPSL, rapidly increasing levels of serum lipids and continuous low levels of platelets were observed 2 weeks after injection, while these effects were not observed in rabbits receiving 5 and 1 mg/kg MPSL. Since acute hyperlipidemia and a hypercoagulable state in

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

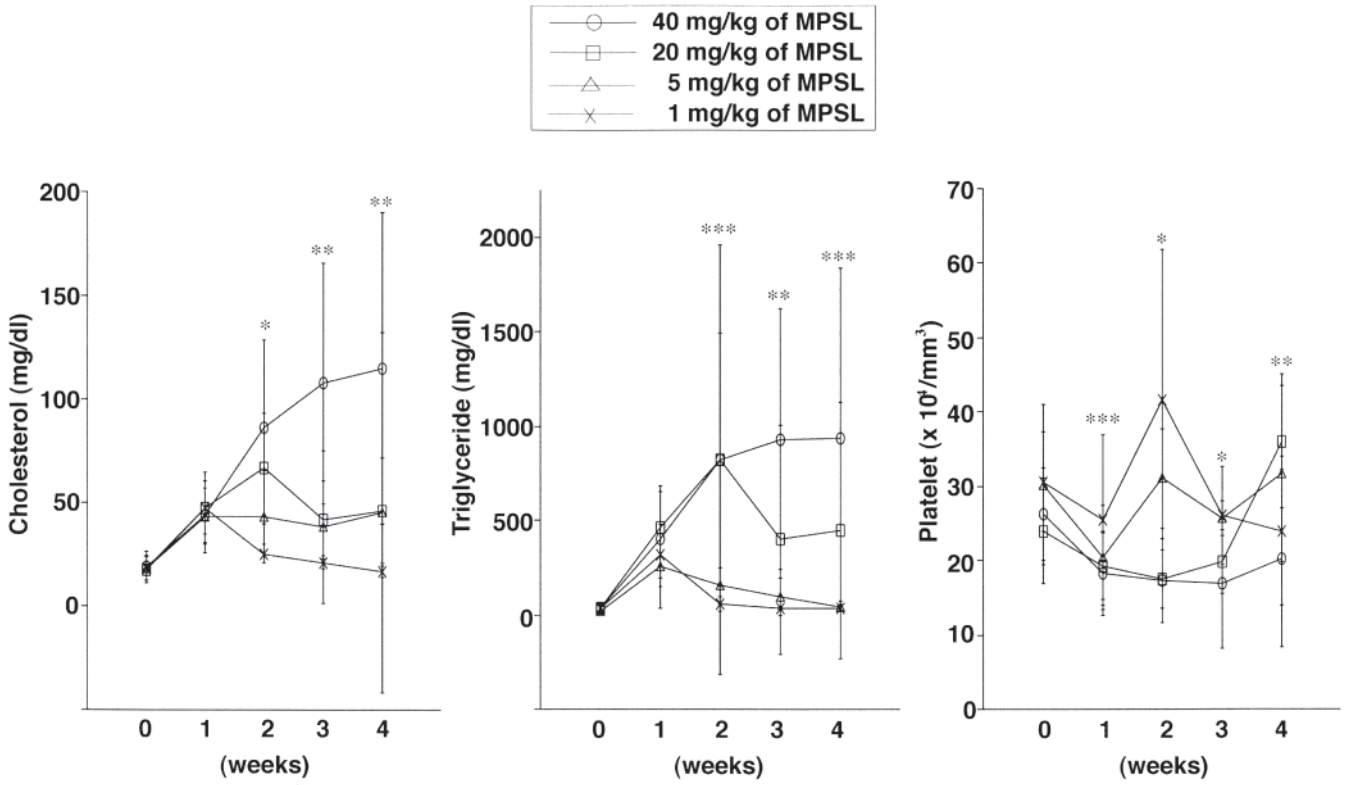

Figure 3. Sequential changes in levels of cholesterol, triglycerides, and platelets in each study group. *Significant difference: 40 mg/kg vs 1 and 5 mg/kg of MPSL, 20 mg/kg vs 1 and 5 mg/kg MPSL, or 20 vs 1 mg/kg MPSL. **Significant difference: 40 mg/kg vs 1, 5, and 20 mg/kg MPSL, or 40 mg/kg vs 5 and 20 mg/kg MPSL. ***Significant difference: 40 mg/kg vs 1 and 5 mg/kg MPSL, or 40 vs 1 mg/kg MPSL.

the early period after corticosteroid injection have been considered to be important for the development of ON in this model[10,12], we speculate that a positive relationship between the dose of corticosteroids and the prevalence of ON may be attributed to the effects of corticosteroids on both the lipid metabolism and the coagulation system.

Regarding histopathological features, we found a difference in the reparative tissues around the ON areas 4 weeks after injection between rabbits receiving 5 mg/kg MPSL and those receiving 20 and 40 mg/kg MPSL. Yamamoto, *et al* reported that the repair process around the ON in rabbits with 20 mg/kg of MPSL was observed at 6 weeks after injection[10], suggesting that the repair process in the rabbits with 5 mg/kg MPSL was formed earlier than that in rabbits with 20 mg/kg MPSL. Recently, Radke, *et al* described the expression of vascular endothelial growth factor (VEGF) in the femoral head in ON[16], suggesting the association of angiogenic proteins with the repair process of ON. The inhibitory effect of corticosteroids on expression of VEGF has been reported[17]; corticosteroid-mediated suppression of the reparative response may have delayed the timing of repair in the rabbits receiving 20 and 40 mg/kg MPSL. The early timing of repair may contribute to the subsequent early remodeling of ON. We therefore speculate that the reduction of corticosteroid dose may have some beneficial effects, not only on the development of ON, but also on the repair process after development of ON. Considering the various conditions of the underlying diseases, however, it is difficult to perform appropriate reduction of corticosteroids for all patients. We suppose that it may be important to identify patients who may be at high risk for ON before administration of corticosteroids.

High-dose corticosteroid does not always cause ON in humans or animals, suggesting an interindividual difference in susceptibility. Our study suggests the possibility that rabbits with low susceptibility to corticosteroids may also develop ON in association with the increased dose of corticosteroids. Recently, the activity of hepatic cytochrome P450 3A4, which metabolizes corticosteroids, has been suggested to be associated with the development of ON[18]. Masada, *et al* revealed the inverse relationship between hepatic cytochrome P450 3A activity and the prevalence of ON in rabbits[18], suggesting the possibility that development of ON may be avoided by reducing corticosteroid doses in patients with low hepatic cytochrome P450 3A activity. This finding gives us hope for development of precisely calculated corticosteroid treatments. We believe that the dose of corticosteroids should be reduced in the treatment of various diseases as much as possible, in order to minimize the development of ON. Even after the development of ON, our data

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

suggest the association of corticosteroids with the reparative process. Considering the inhibitory effects of corticosteroids on the reparative process, early surgical interventions may be needed for preserving affected femoral heads.

REFERENCES

1. Mont MA, Jones LC, Hungerford DS. Nontraumatic osteonecrosis of the femoral head: ten years later. J Bone Joint Surg Am 2006;88:1117-32.
2. Mont MA, Hungerford DS. Non-traumatic avascular necrosis of the femoral head. J Bone Joint Surg Am 1995;77:459-74.
3. Nagasawa K, Tada Y, Koarada S, et al. Very early development of steroid-associated osteonecrosis of femoral head in systemic lupus erythematosus: prospective study by MRI. Lupus 2005;14:385-90.
4. Griffith JF, Antonio GE, Kumta SM, et al. Osteonecrosis of hip and knee in patients with severe acute respiratory syndrome treated with steroids. Radiology 2005;235:168-75.
5. Mok CC, Lau CS, Wong RW. Risk factors for avascular bone necrosis in systemic lupus erythematosus. Br J Rheumatol 1998;37:895-900.
6. Mont MA, Glueck CJ, Pacheco IH, Wang P, Hungerford DS, Petri M. Risk factors for osteonecrosis in systemic lupus erythematosus. J Rheumatol 1997;24:654-62.
7. Lieberman JR, Roth KM, Elsissy P, Dorey FJ, Kobashigawa JA. Symptomatic osteonecrosis of the hip and knee after cardiac transplantation. J Arthroplasty 2008;23:90-6.
8. Lieberman JR, Scaduto AA, Wellmeyer E. Symptomatic osteonecrosis of the hip after orthotopic liver transplantation. J Arthroplasty 2000;15:767-71.
9. Colwell CW Jr, Robinson CA, Stevenson DD, Vint VC, Morris BA. Osteonecrosis of the femoral head in patients with inflammatory arthritis or asthma receiving corticosteroid therapy. Orthopedics 1996;19:941-6.
10. Yamamoto T, Irisa T, Sugioka Y, Sueishi K. Effects of pulse methylprednisolone on bone and marrow tissues: corticosteroid-induced osteonecrosis in rabbits. Arthritis Rheum 1997;40:2055-64.
11. Miyanishi K, Yamamoto T, Irisa T, et al. A high low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio as a potential risk factor for corticosteroid-induced osteonecrosis in rabbits. Rheumatology Oxford 2001;40:196-201.
12. Motomura G, Yamamoto T, Miyanishi K, Jingushi S, Iwamoto Y. Combined effects of an anticoagulant and a lipid-lowering agent on the prevention of steroid-induced osteonecrosis in rabbits. Arthritis Rheum 2004;50:3387-91.
13. Hedri H, Cherif M, Zouaghi K, et al. Avascular osteonecrosis after renal transplantation. Transplant Proc 2007;39:1036-8.
14. Wing PC, Nance P, Connell DG, Gagnon F. Risk of avascular necrosis following short term megadose methylprednisolone treatment. Spinal Cord 1998;36:633-6.
15. Assouline-Dayan Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. Semin Arthritis Rheum 2002;32:94-124.
16. Radke S, Battmann A, Jatzke S, Eulert J, Jakob F, Schütze N. Expression of the angiomatrix and angiogenic proteins CYR61, CTGF, and VEGF in osteonecrosis of the femoral head. J Orthop Res 2006;24:945-52.
17. Nauck M, Roth M, Tamm M, et al. Induction of vascular endothelial growth factor by platelet-activating factor and platelet-derived growth factor is downregulated by corticosteroids. Am J Respir Cell Mol Biol 1997;16:398-406.
18. Masada T, Iwakiri K, Oda Y, et al. Increased hepatic cytochrome P4503A activity decreases the risk of developing steroid-induced osteonecrosis in a rabbit model. J Orthop Res 2008;26:91-5.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org