ACTA
ORTHOPAEDICA
et
TRAUMATOLOGICA
TURCICA

Author's translation
Acta Orthop Traumatol Turc 2007;41(1):58-63

The effect of steroid use on the pathogenesis of avascular necrosis of the femoral head: an animal model

Steroid kullanımının femur başı avasküler nekrozu patogenezindeki yeri: Deneysel hayvan modeli

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Amaç: Sağlıklı ve serum hastalığı oluşturulmuş sıçanların femur başlarında steroid tedavisinin etkileri araştırıldı.

Çalışma planı: Ağırlıkları 250-300 gr arasında değişen 30 adet Sprague-Dawley cinsi sıçan eşit sayıda üç gruba ayrıldı. A grubundaki deneklere iki hafta arayla iki kez steril insan serumu (10 ml/kg) periton içine verilerek serum hastalığı oluşturuldu. Bunu izleyen iki haftadan sonra üç gün 40 ml/kg/gün metilprednisolon verildi. B grubundaki deneklere üç gün yalnızca 40 mg/kg/gün metilprednisolon verildi. C grubundaki (kontrol) sıçanlara A grubundaki işlemlerin aynısı yalnızca serum fizyolojik ile uygulandı. Her grupta işlem bitiminden iki hafta sonra deneklerin yaşamı sonlandırıldı ve femurlar çıkarıldı. Histolojik incelemeler için sol femurlarda aksiyel, sağ femurlarda sagittal kesitler hazırlandı. Işık mikroskopisi ile vaskülit, kanama ve hücre morfolojisindeki değişiklikler incelendi.

Sonuçlar: Histolojik incelemelerde, A grubu deneklerde kemik iliğinde belirgin bir azalma, nekroz alanları, yaygın kanama alanları, lakünalar ve küçük osteonekroz alanları izlendi. Vaskülit bulguları olarak arteriol duvarı düz kasında nekroz, tunika media tabakasında dejeneratif değişiklikler göze çarpmaktaydı. B grubunda daha çok kemik iliğine ait hücresel değişiklikler görüldü. Kemik trabeküllerinde osteonekroz bulgularına rastlanmadı. C grubunda intramedüller kanama dışında herhangi bir patolojiye rastlanmadı.

Çıkarımlar: Steroid kullanımı kemik nekrozunun ana nedeni olmamasına karşın, var olan etkenleri tetikleyerek femur başı avasküler nekrozuna yol açmaktadır.

Anahtar sözcükler: Femur başı nekrozu; osteonekroz; sıçan; steroid/yan etki.

Objectives: We investigated the effect of corticosteroid treatment on the femoral head of healthy and serum disease-induced rats.

Methods: Thirty Sprague-Dawley rats weighing 250-300 g were divided into three groups equal in number. In group A, serum disease was induced by two intraperitoneal injections of sterile human serum (10 ml/kg), interspersed with a two-week interval. The rats were then treated with methylprednisolone (40 mg/kg/day) for three consecutive days two weeks after the last injection. In group B, the animals received only methylprednisolone (40 mg/kg/day) for three days. In group C (controls), the same procedures were applied as those in group A, but with saline solution alone. All the rats were sacrificed two weeks after the last procedure. The left and right femora were sectioned axially and sagittally, respectively, to be examined under light microscopy with respect to vasculitis, hemorrhage in bone marrow, and changes in cell morphology.

Results: Histological examination showed decreased bone marrow, necrotic areas, diffuse hemorrhage, lacunae, and small areas of osteonecrosis in group A rats. Necrosis in the smooth muscle of the arteriole walls, and degenerative changes in the tunica media were suggestive of vasculitis. In group B, major findings were cellular differentiation of bone marrow, without findings of osteonecrosis in bone trabeculae. In the control group, no pathologic findings were observed other than intramedullary hemorrhage.

Conclusion: Even though it is not the main cause of bone necrosis, steroid administration increases the risk for avascular necrosis of the femoral head.

Key words: Femur head necrosis; osteonecrosis; rats; steroids/adverse effects.

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Received: 14.06.2006 **Kabul Accepted:** 22.11.2006

Fremont A. Chandler reported that bone anoxia causes a symptom complex which he named as femoral head infarction characterized with pain, functional loss, and bone collapse. Avascular necrosis of femoral head (ANFH) has been defined as regional osseous tissue death and necrosis due to loss of vascular support. Traumatic femoral head osteonecrosis is seen subsequent to femoral head fractures and traumatic hip dislocations. Atraumatic avascular necrosis of femoral head is more often seen as a bilateral entity in youngsters. Patients in this group are still problematic with respect to treatment planning due to their poor prognoses. Many diseases have been reported in the pathogenesis of avascular necrosis of femoral head and various theories have been emphasized. Corticosteroid usage, alcoholism, hemoglobinopathies, disbarism, depot diseases may lead to ANFH. Among these osteonecrosis due to steroid usage constitutes an important group. Steroids are used widely in the treatment of nerve lacerations, replantations and connective tissue disorders. Different percentages of ANFH encountered in these diseases demonstrate that steroids do not always act through the same mechanism of action. The most frequently emphasized theories are accumulation of fat embolies in intraosseous arterioles which could lead to obstruction, coagulopathies, and increased intraosseous pressures which could ensue in avascular necrosis similar to a compartment syndrome. As the source of fat embolisms, fatty liver, higher doses of steroids or stabilization of endogenous lipoproteins have been implicated.[1] Wang et al.[2] demonstrated increments in the shapes and volumes of adipocytes as a result of steroid therapy. Subsequent studies stressed increase in fat as an causative factor in the pathogenesis of obstructive processes. The impact of steroids on bone marrow, osseous tissue and remodelling has not been elucidated fully. Since experimental models used are generally four-legged animals, the hip biomechanics could not be revealed completely.

In this study the effects of steroid therapy on ANFH in healthy rats, those with serum sickness and also control groups were investigated. With histological examinations, structural changes in femoral heads due to sterod usage have been put forth.

Material and method

In the present study 30 Sprague-Dawley rats weighing 250-300 g were used. The rats were divided equally in three groups including a control group. Ten rats in Group A received 10 ml/kg human serum intraperitoneally at 2 week-intervals. After successive 2 weeks 40 ml/kg/d methylprednisolone for 3 days was administered. Group B rats received daily doses of only 40 mg/kg prednisolone for 3 days. For Group C rats (control) the same procedures with only physiologic saline were repeated. The saline solution (10 ml/kg) was given intraperitoneally at 2- week intervals, and then at dosages of 40 mg/kg/d for 3 days successively. Two weeks after the completion of the procedures, the rats were sacrificed.

After termination of the experiment, femoral heads of rats were excised, and fixed with 10 % formaldehyde. For the decalcifications of right femurs 5 % formic acid, and for left femurs trichloroacetic acid were used. The samples were embedded in paraffine blocks (right femurs longitudionally, and left femurs transversely) and examined and monitored histologically. Five 5 μm- thick paraffine sections obtained were stained with hematoxylene-eosin, saphranin-fast green, and trichromic Masson stain. Prepared slides were examined under light microscope, and photographed.

Results

Any microscopic and gross deformity and collapse were not detected in femoral heads of rats. The results of microscopic examinations are shown in Table 1.

Table 1. Histological changes categorized according to groups

Groups	Femoral heads examined (n)	Osteonecrosis		Vasculitis		Cellular changes		Intramedullary bleeding		Normal findings	
		n	%	n	%	n	%	n	%	n	%
A	20	18	90	18	90	20	100	16	80	0	0
B	20	3	15	4	20	14	70	15	75	6	30
C	20	0	0	0	0	0	0	2	0	18	90

Histological examinations of samples obtained from Group A rats revealed a marked rarefaction in bone marrows, patchy areas of focal necrosis, and extensive intramedullary hemorrhagic areas. Cellular contours in areas of necrosis were irregular. Abundant lacunes without osteocytes were seen. Besides in some sections, small areas of osteonecrosis and vasculitis were observed. Observed manifestations of vasculitis consisted of necrosis of arteriolar wall smooth muscle, and degenerations in tunica media. Walls of epiphyseal vessels did not show any pathological changes. Intramedullary bleedings seen in areas of dense necrosis suggested old hemorrhagies. Morphological changes were seen in adipocytes in this region, while any finding suggestive of osteogenesis was not encountered. These features were also observed in metaphysis and diaphysis of femurs (Figure 1a, b). Dense areas of necrosis and intramedullary bleeding rarefied towards periphery. In remote areas completely normal bone marrow and trabecular structures were seen, while focal areas of vasculitis and bleeding were observed between normal and osteonecrotic regions.

In Group B, cellular changes relatively peculiar to bone marrow were seen. Osteonecrotic findings in bone trabeculae were not found, while extensive areas of bleedingattracted our attention. Findings of

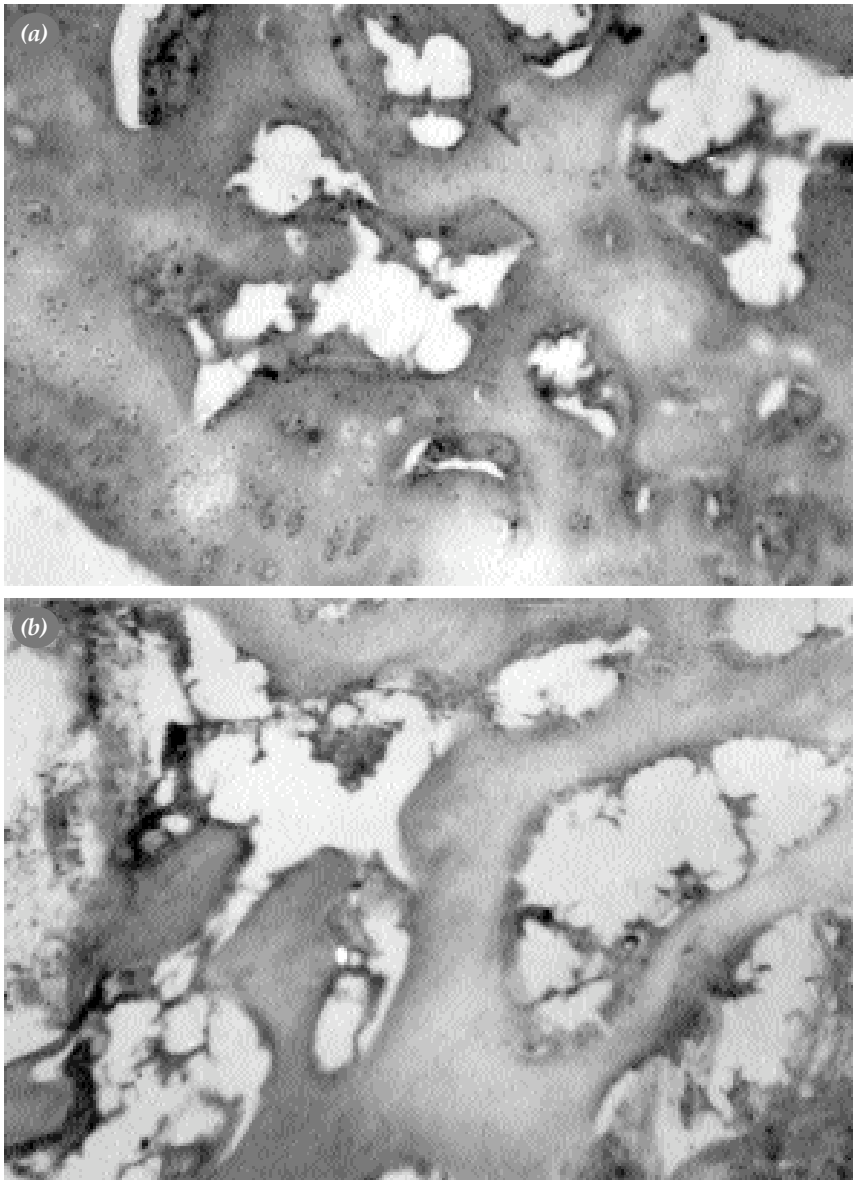

Figure 1. Subject Group A **(a)** (H-E x 16) **(b)** H-E x 82)

vasculitis were encountered in 4 sections, while cellular changes, cytolysis, karyorhexis and karyolysis were observed in 14 sections (Figure 2). In 6 sections any pathological finding was not detected. Intramedullary hemorrhagies were detected in 15, and osteonecrosis in 3 sections. Almost in every section alterations in the morphologies of adipocytes were seen.

In Group C, any abnormality besides intramedullary bleeding was not encountered (Figure 3). In statistical analyses using Student t-tests, any significant difference between groups was not seen ($p < 0.01$).

Discussion

The correlation between ANFH and steroid usage has been conventionally acknowledged. In literature in only 8-10 percent of steroid users osteonecrosis has been reported. Immune systems of steroid user are affected in some way or other. Avascular necrosis is seen in cases where steroids are used for various indications such as connective tissue disorders, rheumatoid arthritis, lupus or organ transplants. However in these cases general state of vasculitis is already present due to the natural course of the disease. In many cases suspicions whether underlying chronic disease or steroid usage could lead to avascular necrosis have

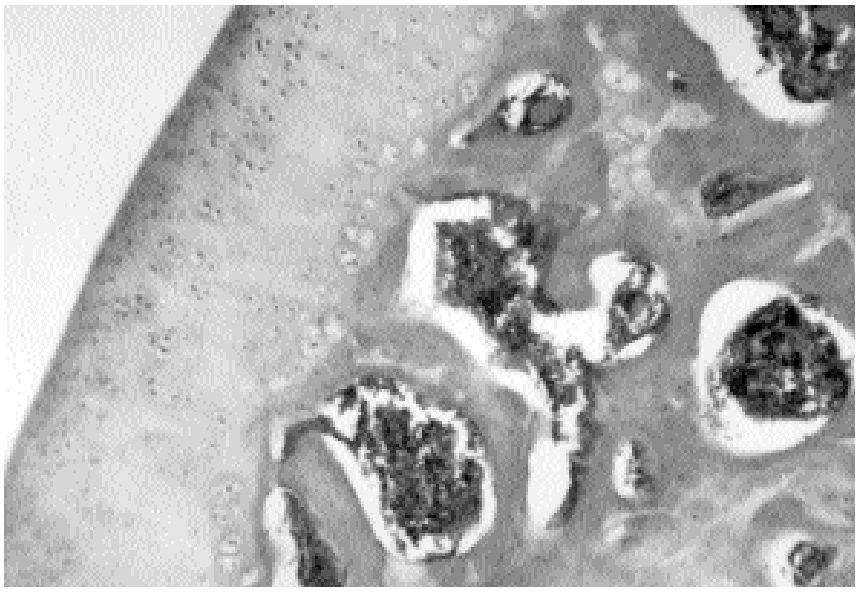

Figure 2. Subject Group B (H-E x 16)

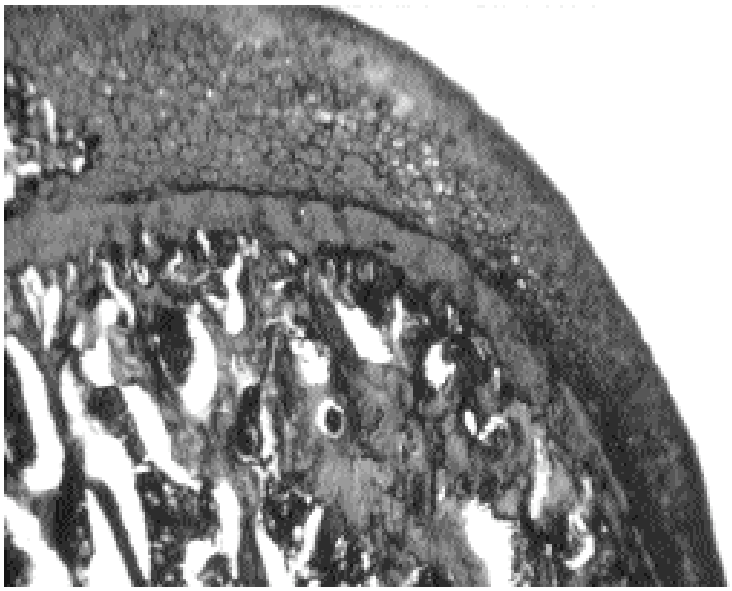

Figure 3. Subject Group C (Saphranin fast green x 16)

not been settled yet.[3,4] Chronic steroid usage does not result in avascular necrosis in cases with renal diseases, while post-transplant incidence of ANFH is increasing rapidly.

In these circumstances what is the causative factor? Organ transplantation or steroid usage ? Adequate number of studies elucidating the role of steroids in the pathogenesis of avascular necrosis has not been conducted in experimental animal models. In animal experiments where higher doses of steroids have been used, investigators encountered fatty degenerations, and vascular changes, however avascular necrosis has not been encountered in any study whatsoever. However osteoporosis was detected in every study.[5-7] In a few studies, experimentally induced osteonecrosis with higher doses of steroid therapy in a diseased background has been attempted. Matsui et al.[8] administered higher doses of steroids to rabbits with hypersensitivity vasculitis (acute serum disease) induced by repetitive injections of horse serum, and reported occurrence of osteonecrosis.

In a rabbit model immunized with anti-DNA antibody, a lupus-like syndrome was induced. Then histological response to steroids was investigated, and intravascular coagulation and various degrees of obstructive processes were found in a rate of 80 % after immunization.

Our study is based on investigations of short-term histological changes in rats. In rats with induced serum disease, significantly higher number of varying morphological alterations was detected. In Group B rats where only prednisolon was administered, microscopic examinations of femoral heads revealed cellular changes in 14, and intramedullary bleedings in 15 rats. Emerging bleeding was anticipated to result in increase in intraosseous tissue pressures leading to osteonecrosis, on the contrary the presence of only three cases of avascular necrosis is noteworthy. Kenzore stated that intraosseous administration of steroids as an antitumoral therapy did not lead to necrosis, and avascular necrosis was not seen in steroid users for head trauma, and questioned the correlation between steroid usage and bone necrosis all of which are in accordance with our argument. The same author found that corticosteroids halted DNA synthesis, and on the contrary RNA synthesis was maintained without any interruption in protein synthesis and cellular viability.[9, 10] Corticosteroids cause osteoporosis which indicates viability of the bone tissue. All of these demonstrate that corticosteroid usage is not the only causative factor for the development of osteonecrosis. In a study performed by Korompilias et al.[11] with rabbits, they had observed pathological changes in 80 % of animals with induced vasculitis who received steroids subsequently. However in the same study, in only two of ten rabbits treated with only methyl prednisolone-prednisolone, blood clots were seen in veins of metaphyseal regions outside the osseous tissue. Solomon [12] explained osteonecrosis occurring in hypercorticoidism with fatty degeneration of liver, hyperlipidemia, and fat embolisms all of which are frequently seen in these cases. In Group B rats osteonecrosis was not seen, however serious pathological changes as large areas of bleeding, cytolysis, karyorhexis, and karyolysis were frequently encountered. Steroid usage affects bone seriously. However in the presence of vasculitis, steroid usage leads to severe histopatological changes as in Group A. These changes result in osteonecrosis. Under the light of all these data, we can say that findings revealed in these animal experiments confirm Kenzora's "Accumulative Cell Stress" theory.

In our opinion, steroid usage is risky especially in patients with immune system abnormalities. Development of osteonecrosis in seemingly healthy patients with steroid usage should mandate investigation for an immune or hematogenous etiology.

References

1. Lieberman JR, Berry DJ, Mont MA, Aaron RK, Callaghan JJ, Rajadhyaksha AD, et al. Osteonecrosis of the hip: management in the 21st century. Instr Course Lect 2003;52: 337-55.
2. Wang GJ, Sweet DE, Reger SI, Thompson RC. Fat-cell changes as a mechanism of avascular necrosis of the femoral head in cortisone-treated rabbits. J Bone Joint Surg [Am] 1977;59:729-35.
3. Fisher DE, Bickel WH, Holley KE, Ellefson RD. Corticosteroid-induced aseptic necrosis. II. Experimental study. Clin Orthop Relat Res 1972;(84):200-6.
4. Jaffe WL, Epstein M, Heyman N, Mankin HJ. The effect of cortisone on femoral and humeral heads in rabbits. An experimental study. Clin Orthop Relat Res 1972;(82):221-8.

5. Cruess RL, Ross D, Crawshaw E. The etiology of steroid-induced avascular necrosis of bone. A laboratory and clinical study. Clin Orthop Relat Res 1975;(113):178-83.
6. Felson DT, Anderson JJ. Across-study evaluation of association between steroid dose and bolus steroids and avascular necrosis of bone. Lancet 1987;1:902-6.
7. Jones JP Jr. Intravascular coagulation and osteonecrosis. Clin Orthop Relat Res 1992;(277):41-53.
8. Matsui M, Saito S, Ohzono K, Sugano N, Saito M, Takaoka K, et al. Experimental steroid-induced osteonecrosis in adult rabbits with hypersensitivity vasculitis. Clin Orthop Relat Res 1992;(277):61-72.
9. Kenzora JE, Glimcher MJ. Accumulative cell stress: the multifactorial etiology of idiopathic osteonecrosis. Orthop Clin North Am 1985;16:669-79.
10. Kenzora JE, Glimcher MJ. Pathogenesis of idiopathic osteonecrosis: the ubiquitous crescent sign. Orthop Clin North Am 1985;16:681-96.
11. Korompilias AV, Ortel TL, Urbaniak JR. Coagulation abnormalities in patients with hip osteonecrosis. Orthop Clin North Am 2004;35:265-71.
12. Solomon L. Mechanisms of idiopathic osteonecrosis. Orthop Clin North Am 1985;16:655-67.