**INTRODUCTION:**
The cause of steroid-induced osteonecrosis (ON) of the femoral head is thought to be blood flow impairment, but true mechanisms have not yet been understood. On the other hand, apoptosis, a particular type of cell death and defined as programmed cell death, commonly occurs in the developing embryo, in normal healthy adult tissues, and also in pathological settings, and it involves in ischemic heart diseases and ischemic cerebrovascular disorders which are induced by the decrease of local blood flow or by hypoxia. It is of interest that apoptosis is related to ischemia, and apoptosis could also be involved in the establishment of ON of the femoral head.

In the present study, apoptosis in steroid-induced ON was investigated using our rabbit model.

**METHODS:**
Animals and treatments: 44 adult female Japanese White rabbits (mean body weight: 3.5 kg) were used. Among the 44 rabbits, 35 were divided into 3 groups. Group A (n = 10) were untreated controls and had a subsequent 8-week no treatment period. Group B (n = 15) were i.m. injected with 4 mg/kg body weight of methylprednisolone once a week for 4 weeks. Group C (n = 10) received the same 4-week treatment and had a subsequent 8-week no treatment period. For light microscopic examinations, tissue samples were obtained from both the femur and humerus. The specimens were stained with hematoxylin and eosin (H-E). ON were determined when empty lacunae or pyknotic nuclei of osteocytes were present diffusely in the bone trabeculae, and they were accompanied by necrosis of the surrounding bone marrow hematopoietic cells including adipocytes. Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick end labeling (TUNEL) was used to detect fragmented DNAs which are known to involve in the establishment of ON of the femoral head.

Quantitative analysis of TUNEL-positive cells: Among the non-ON of Groups A, B and C, numbers of TUNEL-positive cells were not significantly different. However, in the areas of Group B, the positive cell number was significantly larger in the surrounding area of ON than in the center of ON and in non-ON areas (p<0.001). In Group C, the positive cell number did not differ among these 3 areas.

Electron microscopic findings: In the center of ON, most cells were markedly swelled, and there were typical necrotic findings. On the other hand, in Group B, nuclei were condensed and fragmented, and the cytoplasms frequently contained vacuoles in the cells which showed chromatin condensation and located in the surrounding area of ON.

**RESULTS:**
Histopathological findings and in situ detection of fragmented DNA: In Group A animals, ON were not observed. TUNEL-positive cells were found in all specimens, but there were only 3–4 cells among the bone marrow cells in a view of X200. In Group B, ON were detected in 11 rabbits in the area between the metaphysis and diaphysis of the proximal femur, in 3 rabbits in the metaphysis of the distal femur, and in 5 rabbits in the metaphysis of the proximal humerus. ON were stained with eosin of H-E staining, and all bone marrow cells including fat cells in the central part of the ON showed necrosis. In addition, empty lacunae were found in the bone trabeculae. TUNEL was performed using serial sections, and TUNEL-positive cells were not present in the center of ON, but they were accumulated in the surrounding area of the lesion. In the area containing TUNEL-positive cells, there were many cells which nuclei had chromatin condensation and which were strong-stained with hematoxylin. TUNEL-positive cells were found in Group C, ON were found as in Group B, but at the same time, in the surrounding area of ON, there was appositional bone formation around dead bone which was associated with granulation tissues, and there were no TUNEL-positive cells.

**DISCUSSION:**
Several mechanisms of steroid-induced ON have been proposed, and they explain that ischemia in the bone finally leads to ON. On the other hand, recent studies revealed the involvement of apoptosis into ischemic cell death in the brain as well as ischemic heart diseases such as acute myocardial infarction, and relationship between apoptosis and local ischemia or reperfusion disorders has attracted a great deal of attention. Apoptosis would also occur in ON which is thought to be induced by ischemic condition. In the present study, TUNEL-positive cells were localized in the area surrounding the ON of Group B, and TUNEL-positive cells were rarely found in Group C. This showed that apoptosis involves in the early stage of steroid-induced ON.

This finding will have an important implication to future studies on the pathology and development mechanism of this disease, as well as in the development of new treatment and preventive methods.