Introduction

Osteonecrosis (ON) is known to occur in patients who have received corticosteroids for the treatment of such underlying diseases as systemic lupus erythematosus, rheumatoid arthritis and leukemia. Its precise etiology remains controversial, and many experimental animal studies using corticosteroids have suggested a possible pathogenesis of corticosteroid-induced ON, such as an increased size of marrow fat cells, an accumulation of lipid within the osteocytes, and fat emboli. Such intraosseous changes have not yet been compared between corticosteroid-treated animals with ON and those without ON.

Recently, prominent lipid transport has been reported as an etiology of ON, and a higher lipid deposition was reported in human ON femoral head. In bone tissue, such lipid storage is one of the major functions of marrow fat cells. This study was designed to determine whether the size of marrow fat cells differed between the corticosteroid-treated rabbits with ON and those without ON.

Methods

Animals. Adult male Japanese white rabbits (Kyudo, Tosu, Japan), weighing from 3.5 kg to 3.9 kg, were used. All experiments were reviewed by the Common Ethics Committee for Animal Experiments at our university.

Treatment. Forty-eight rabbits were intramuscularly injected once with 20 mg/kg of methylprednisolone acetate (MPSL, Upjohn, Tokyo, Japan), and 6 rabbits were injected once with physiologic saline as a control. All rabbits were killed 4 weeks after the injection, and both sides of the femur and humerus were histopathologically examined for the presence of ON.

Evaluation of ON. ON was blindly assessed by three authors (K.M., T.Y., T.I.). ON was determined based on the diffuse presence of empty lacunae or pyknotic nuclei of osteocytes in the bone trabeculae, accompanied by the presence of either fat emboli or thrombi.

Statistical Analysis. The prevalence of ON was 37 rabbits out of 48 corticosteroid-treated rabbits with ON and those without ON. The data on the fat-cell size were given as the mean ± SD. The size of marrow fat cells were compared among the rabbits with ON, those without ON and the control rabbits using Scheffe’s test. The prevalence of both fat emboli and thrombi was also determined in each rabbit.

Results

Prevalence of ON. The incidence of ON was 37 rabbits out of 48 corticosteroid-treated rabbits (77%). No ON lesions could be seen in any of the control rabbits.

Histopathologic Features. ON lesion showed an accumulation of bone marrow cell debris and bone trabeculae demonstrating empty lacunae.

Fat emboli were noted in the intraosseous vein in 7/37 ON+ rabbits (19%) and 1/11 ON- rabbit (9%). No fat emboli could be seen in any of the control rabbits. A thrombus was noted in the intraosseous arteriole in 1/37 ON+ rabbits (3%), however, it was not seen in either the ON- rabbits or the control rabbits. The prevalence of both fat emboli and thrombi showed no significant difference among the 3 groups.

Measurements of the size of marrow fat cells. The size of the marrow fat cells increased significantly more in the rabbits with ON (58.7±7.1 micrometers) than in the control rabbits (38.3±3.3 micrometers, P < 0.0001) or the control rabbits (P < 0.00001), and more in the rabbits without ON than in the control rabbits (P < 0.05).

Discussion

In conclusion, the marrow fat cells were almost homogeneous in size, in which the space for the bone marrow hematopoietic cells and vascular channels seemed to be preserved. In the rabbits without ON, some of the marrow fat cells increased in size and were observed to contain enlarged intracytoplasmic ovoid lipid droplets, however, the space for the bone marrow hematopoietic cells and vascular channels was relatively well preserved. In the rabbits with ON, the number of the enlarged marrow fat cells increased, while some fat cells remained small in size. As a result, considerable variability was observed in the marrow fat-cell diameter. The bone marrow hematopoietic cells decreased in number, and their space was found to be replaced by the enlarged marrow fat cells, and the vascular channels also appeared to be compressed (Figure 1).

Figure 1. Histomorphometric changes of the marrow fat cells in rabbits. (A) In the control rabbits, the marrow fat cells are almost homogeneous in size, in which the space for the bone marrow hematopoietic cells and vascular channels seems to be preserved. (B) In the rabbits without osteonecrosis, some of the marrow fat cells increase in size, however, the space for the bone marrow hematopoietic cells and vascular channels is relatively well preserved. (C) In the rabbits with osteonecrosis, the number of enlarged marrow fat cells increases. The bone marrow hematopoietic cells decrease in number, and their space has been replaced by the enlarged marrow fat cells.

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