

Early Pathological Changes in Mice with Corticosteroid Induced Osteonecrosis of the Femoral Head - A Preliminary Study -

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INTRODUCTION:

Osteonecrosis of the femoral head (ONFH) is a serious complication associated with long-term corticosteroid therapy, particularly in patients with autoimmune and hematologic diseases. ONFH is a pathophysiological and clinical condition that is due to death of the cells in the bone marrow, progressive collapse of the femoral head, often necessitating total hip arthroplasty. Recently, we established a corticosteroid induced murine model by inserting slow-release prednisolone pellets into subcutaneous tissue (PRED group). In this study, we evaluate early changes of oxidative stress, glucose metabolism, and lipid metabolism between the PRED group and a SHAM group receiving placebo pellets, using histomorphometric evaluation, and PET/CT analysis.

METHODS:

This animal study was approved by our Institutional Animal Care and Use Committee and followed institutional guidelines for the care and use of laboratory animals. We prepared eight 12-week-old Balb/C male mice and randomized the mice into either the SHAM control or the Prednisolone treatment (PRED) group. The mice in the PRED group underwent insertion of a 2.5 mg slow-release prednisolone pellet subcutaneously in the dorsal spine. At 1-week and 6-weeks, body weight, bone mineral density (BMD), histological evaluation (hematoxylin and eosin (HE) staining for empty lacunae, tartrate-resistant acid phosphatase (TRAP) staining for osteoclasts, nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) staining for oxidative stress), and FDG-PET/CT data(%ID/cc) of the femoral bone were evaluated between two groups. Two-way ANOVA with Sidak's multiple comparison test was used for multiple group comparisons. The Mann-Whitney U test was used to compare two groups. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using Prism 9 (GraphPad Software, San Diego, CA, USA).

RESULTS SECTION:

Mean body weight in the PRED group decreased from 31.3g to 28.7g at 1-week after surgery.

The mean lumbar spine BMD in the PRED group significantly decreased at 6-week compared to the SHAM group (PRED:87.7mg/cm², SHAM:98.7mg/cm²) (Fig. 1a). The number of empty lacunae in the PRED group was significantly higher than that in the SHAM group at 6-weeks (Fig 1b).

The number of osteoclasts (TRAP stain) in the PRED group was significantly higher than that in the SHAM group at week 6 (Fig. 1c). NOX2 expression in the femoral head was slightly (NS) higher in the PRED group than that in the SHAM at 1 and 6-week (Fig. 1d). According to the results of FDG-PET/CT, the mean value of %ID/cc in 1-week SHAM group was 3.38, and that of 6-week was 4.38. The mean value of %ID/cc in 1-week PRED group was 2.64, and that of 6-week was 4.50. There were no significant differences between two groups and time points (Fig. 2).

DISCUSSION:

This study evaluated early changes in the corticosteroid-associated ONFH mice models using histological evaluation and FDG-PET/CT.

In histological evaluation, although there was no significant difference between the SHAM and PRED group at 1-week, the number of empty lacunae at 6-week in the PRED group increased significantly compared to that of the SHAM group. These results indicate osteocyte apoptosis and structural deterioration at 6-week in the PRED group. Prior to these changes, the results of NOX2 staining showed an increase in oxidative stress, as indicated by sustained NOX2 expression in the femoral head. In addition, TRAP staining showed osteoclast activity increased significantly at 6-week in the PRED group, consistent with bone resorption and remodeling. In our PET/CT results, there was no significant difference between 1-week SHAM and 1-week PRED group, however the mean value of %ID/cc in 1-week PRED group was decreased compared to 1-week SHAM.

Future studies with a larger sample size may help clarify whether glucose uptake on PET/CT decreases at an earlier time point. Although signal changes in MRI or radiographs typically appear 2-3 months after steroid administration in ONFH patients, this study suggests that glucose metabolic changes within the femoral head can already be observed as early as 6 weeks after continuous corticosteroid administration or at an earlier stage during disease onset and progression that precedes histological changes (empty lacunae, oxidative stress, osteoclasts). Evaluating such early changes may contribute to the early detection and treatment of ONFH.

SIGNIFICANCE/CLINICAL RELEVANCE:

This study detected the early changes of osteonecrosis in murine model using PET/CT and histological evaluation. Identifying the early changes of ONFH could contribute to the earlier diagnosis and potential interventions to prevent the progression of this disease.

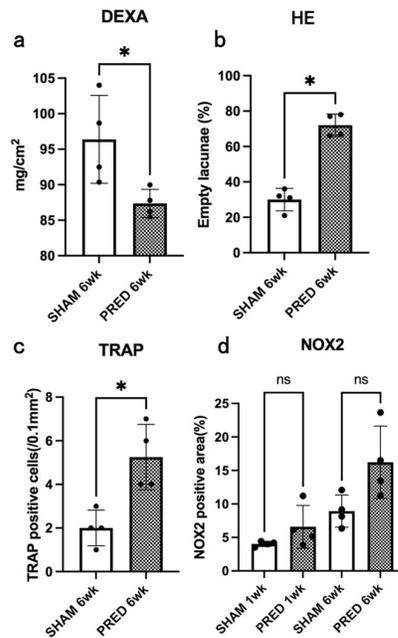


Figure 1

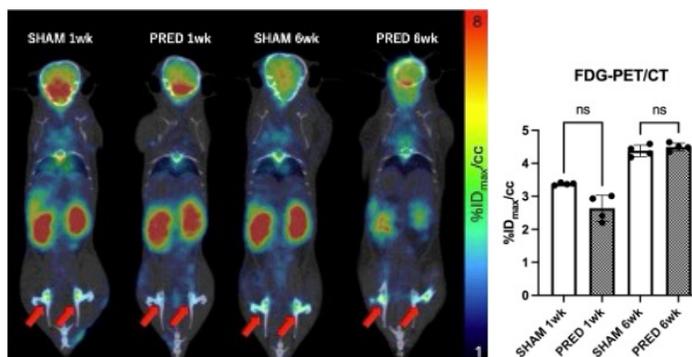


Figure 2