Lumbar Spine of Spontaneously Developing Osteoarthritis Mouse (STR/ort mouse) - Radiological and Histological study-

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INTRODUCTION: The STR/ort strain of mouse, an inbred strain derived from STR/1N, has been reported to be an acceptable animal model for studying the development of osteoarthritis (OA) [1]. About 85% of all male mice spontaneously develop OA in the medial tibial plateau of the knee joint at 7-9 months of age. Importantly, histopathological changes in the lesions of the STR/ort mouse closely resemble those of human OA. These age-related OA changes were also found in the ankle joint of STR/ort mouse, suggestive of a systemic basis for the disease process.

A biochemical feature of OA cartilage is the degradation of the extracellular matrix (ECM) induced by the local production of pro-inflammatory cytokines and matrix-degrading enzymes; this is identical to what is seen in the degenerated intervertebral disc (IVD). To date, several animal models of a naturally-occurring IVD degeneration have been reported [2]. It remains unknown whether STR/ort mice show degenerative changes in the IVD in conjunction with the development of OA.

The purpose of this study was to determine if lumbar spondylotic changes with spontaneous age-related IVD degeneration can be seen in STR/ort mice. In the present study, we have examined the radiologic and histological changes of the lumbar spine of STR/ort mice in a cross-sectional study. In a computerized tomography (CT) analysis study, the lumbar spines of 32-week-old STR/ort mice were used to quantify the parameters of the lumbar spinal column.

METHODS: Animals: Twenty STR/ort mice (4, 8, 12, 40 week-old [n = 5, respectively], male) were used in a cross-sectional study. Eight CBA mice (4, 8, 12, 40 week-old [n = 2, respectively], male) were used for controls. Five STR/ort and CBA mice (32-week-old, male) were used in a CT analysis study.

Cross sectional study: Lateral radiographs (soft-X ray) of the spine (25 animals) were obtained after intramuscular administration of nembutal. After sacrifice and following the measurement of body weight, lumbar spines were removed and processed for histological analyses. Lateral radiographs of lumbar disc spaces (L1-L2, L2-L3, L3-L4, L4-L5, L5-L6) were evaluated by three orthopedic surgeons for the presence of the following features: disc wedging, the presence of osteophytes, and ligament calcification.

Histological analysis: The lumbar spines were fixed in 4% paraformaldehyde, decalcified, embedded in paraffin, sectioned mid-sagittally at 5 μm, and stained with either hematoxylin and eosin, or Safranin-O. For histological grading of the IVD, the observer analyzed the histologic sections and graded them using a previously reported protocol to evaluate tissue structure and cellularity of the IVD tissues [3]. To evaluate the extent of ossification in the cartilaginous end plate (CEP), the following grading scale was used: grade 0 - no ossification; grade 1 - moderate ossification (less than 50% in the CEP); and grade 3 - moderate ossification in STR/ort mice compared to CBA mice (8W: CBA, 2.6 ± 0.5, STR, 2.0 ± 0.5; 12W: CBA, 3.0 ± 0.5, STR, 2.8 ± 0.5). There was no significant difference between CBA and STR mice.

CT analysis study: Sagittal images of lumbar spines were obtained using X-ray CT (LaTheta®, Aloka). Three-dimensional images were constructed by eight serial sagittal sections in the transverse diameter of the lumbar spine. On the mid-sagittal image of each sample, the following parameters were determined by NIH image analysis software: (A) anteroposterior diameter of the spinal canal; (B) diameter of the vertebrae; and (C) diameters of the intervertebral disc space. The samples used in CT analysis study were also processed for histological analysis.

Statistical Analysis: The significance of differences among means of data on the CT analysis study was analyzed using a 1-way ANOVA and Fisher protected least significant difference as a post hoc test. The Kruskal-Wallis and Mann-Whitney U tests were used to analyze nonparametric data (i.e., histology grading for each parameter).

RESULTS: Cross sectional studies:

Body weight: The body weight of STR/ort mice was significantly higher than that of CBA control mice, except for the 4-week-old mice (4W, STR: 14.5 g, CBA: 21.3 g; 8W, STR: 30.0 g, CBA: 25.6 g; 12W, STR: 30.1 g, CBA: 23.9 g; 40W, STR 42.8 g: CBA: 28.1 g).

Radiographic assessment: Osteophyte formation was seen at either the L4/5 or L5/6 IVD of STR/ort mice (4W: 1/5; 8W: 4/5; 12W: 4/5; 40W: 5/5). In CBA control mice, osteophyte formation was observed only in 40-week-old mice (4W: 0/2; 8W: 0/2; 12W: 0/2; 40W: 1/2). Disc wedging was found at either the L4/5 or L5/6 disc of STR/ort mice (4W: 0/5; 8W: 0/5; 12W: 2/5; 40W: 5/5), however disc wedging was not identified in the control CBA mouse. No animals showed ligament calcification in this study.

Histological Analysis: The histology of the IVD tissues, including the annulus fibrosus and nucleus pulposus, in the STR/ort mouse was similar to those of the CBA mouse. No histological features of disc degeneration were found in either STR/ort or CBA mice. There was no significant difference in the IVD histological scores between STR/ort and CBA mice at any age.

In the cartilaginous end plate (CEP) of CBA mouse, slight to moderate ossification was found in 8-week-old mice, and ossification gradually matured in 12, 32 and 40-week-old mice. However, no ossification in the CEP was found in STR/ort mice at 4, 8, 12, and 32 weeks of age. Moderate ossification was found in 40-week-old STR/ort mice. The ossification grading score was significantly higher in CBA mice compared to that of STR/ort mice (8W: CBA, 1.9 ± 0.2, STR, 1 ± 0; 12W: CBA, 2.6 ± 0.5, STR, 1 ± 0; 40W: CBA, 3.0 ± 0, STR, 1.2 ± 0.2; all p<0.05 between CBA and STR).

CT analysis studies: To quantify the parameters of the lumbar spinal column, 32-week-old STR/ort mice and age and sex matched CBA mice were used for CT scans and image analysis. (A) The anteroposterior (AP) diameter of the spinal canal decreased as the spinal level progressed caudally. The spinal canal AP diameter of STR/ort mice did not differ significantly from those of CBA mice. (B) The diameter of vertebrae increased as the spinal level progressed caudally. There was a trend toward larger diameter of vertebrae in STR/ort mice compared with CBA mice. Statistical significance was found at the L3 vertebra (p<0.05). (C) There was also a trend toward smaller diameter disc spaces in STR/ort mice compared to CBA mice. Statistical significance was found at the L5/6 disc space (p<0.01).

DISCUSSION: In the present study, we assessed the radiology and histology of the lumbar spine of STR/ort mice. The incidence of spondylotic changes in the lumbar spine was remarkably higher in the STR/ort mouse compared with that of CBA mice. Although no histological changes in IVD degeneration were found in STR/ort mice or CBA mice, the ossification of the CEP was significantly impaired in STR/ort mice compared to that of CBA mice. In conclusion, STR/ort mice showed spondylotic changes at a high rate than CBA mice, but degenerative IVD changes in STR/ort mice were not remarkable. Further studies on the association of the pathologic changes in the CEP and the lumbar spondylotic changes should be performed.