RECOVERY OF IMMUNE PRIVILEGE IN INTERVERTEBRAL DISC WITH TRANSPLANTATION OF MESENCHYMAL STEM CELLS IN A CANINE DISC DEGENERATION MODEL

Introduction
Transplantation of mesenchymal stem cells (MSCs) can decelerate degeneration in a canine disc degeneration model as detected by radiographic changes and MRI findings in vivo. However, there are many unknown points concerning this new therapy that needs further investigation, such as environmental changes in the intervertebral disc after MSC transplantation or functional analysis of MSCs in the transplanted site [1,2]. Intervertebral discs, nucleus pulposus in particular, possess an immunologic privilege from an anatomical characteristic isolated by host immune system. Takada et al. have reported that expression of Fas ligand(Fasl), which is only found in tissues with isolated immune privilege, is detected in the nucleus pulposus [3]. FasL binds to Fas as a death receptor, which causes apoptosis of the target cell. Thus maintaining this Fas-FasL system is important for function of intervertebral disc. The purpose of this study is to evaluate whether MSCs transplantation have any effect on the Fas-FasL system, using a large animal model for intervertebral disc degeneration model, in conjunction with the process of disc degeneration and regeneration by histological, radiographic, MRI grading assessment, and immunohistochemical analysis.

Materials and Methods
A total of twelve mature beagles were equally divided into 3 groups (Group A: normal control, B: MSCs transplanted, C: disc degeneration model). Disc degeneration was induced in lumbar discs by nucleotomy [4], and MSCs were harvested from the bone marrow. At 4 weeks after the first operation, autologous MSCs were percutaneously transplanted to degenerated discs. At 4, 8 and 12 weeks after the first operation, radiographs and MRI were taken to evaluate disc height and signal changes [5,6]. At 12 weeks after the first operation, histological and biochemical analyses were performed. The harvested spinal columns were stained with H-E and Safranin-O for evaluation. Grading system for disc degeneration by Nishimura was used for evaluation. Immunohistochemical staining was carried out to determine the expression of Fas and FasL. Two pathologists were responsible for counting the total disc cells and FasL and Fas-positive disc cells under 10 high power fields (HPFs; magnification X400) for each of three sections in each group. Finally, the percentage of FasL and Fas positive disc cells of total disc cells was calculated. The proteoglycan content of discs was measured quantitatively using a DMMB assay. Statistical differences were analyzed by ANOVA using the Fisher’s PLSD as a post hoc test.

Results
Radiographic and MRI Grading Assessment: At 4 weeks after the MSCs injection, the mean %DHI of injected discs in the MSC transplanted discs group (Group B) was significantly higher than in the degeneration group (Group C) (Fig.1). This significant difference was maintained during the follow-up. The MRI grading system using Phirmann’s classification also showed significant delay in progression of disc degeneration, suggesting an increase in water content in the nucleus pulposus area in Group B (Fig.2).

Histological assessment: Histology showed relatively preserved inner annular structure in Group B compared to Group C (Grade 2.3 ± 0.9 grade. Group C: 4.0 ± 0.6 grade) (Fig.3).

Proteoglycan Content: The PG content of the nucleus pulposus area was significantly higher in the Group B than Group C.

Immunohistochemistry Assessment: Both Fas and FasL were detected in the cytoplasm of disc cells of the nucleus pulposus area (Fig.4). The FasL positive cells in the nucleus pulposus area decreased significantly in group C, but were restrained significantly in group B (Group A: 27.7 ± 11.1%, Group B: 29.0 ± 14.1%, Group C: 18.4 ± 13.5%) (P<0.05) (Fig.5). The Fas positive cells in the nucleus pulposus area increased significantly in group C (Group A: 20.2 ± 13.8%, Group B: 28.0 ± 10.9%, Group C: 34.6 ± 18.9%) (P<0.05) (Fig.6).

Discussion
Transplantation of autologous MSCs to the canine disc degeneration model resulted in deceleration of disc degeneration. The proportion of FasL positive cells decreased and the proportion of Fas positive cells increased in the disc degeneration group (Group C), whereas FasL positive cells were maintained in the normal control group (Group A) and MSC transplanted disc group (Group B). This suggests that transplantation of MSC may contribute to maintenance of immune privilege in nucleus pulposus, possibly by transplanted MSCs expressing FasL or MSCs stimulating FasL positive cells. With the importance of maintaining FasL positive cells and Fas-FasL system in preventing disc degeneration, the current study implies a new insight in the regenerative effect of MSCs transplantation.

References
4: Iwashina T, et.al.2005,ORS.
5: Masuda K, et.al.Spine2004;30: 5-14

53rd Annual Meeting of the Orthopaedic Research Society

Paper No: 0250