Histological and micro computed tomography (micro CT) features of the intervertebral disc and the endplate after axial compression and distraction of the intervertebral disc in a rabbit model

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ABSTRACT INTRODUCTION:

The endplate is responsible for the nutrition of the disc by allowing diffusion of fluids and nutrients. It has been postulated that changes in endplate permeability maybe responsible for degeneration of the disc. However, another study revealed that the importance of the endplate in disc degeneration maybe debatable. The objective of our study is to assess the histological and micro CT features of the intervertebral disc and the endplate after axial compression and distraction of the intervertebral disc in a rabbit model.

METHODS:

Our method of iatrogenic disc degeneration will be based on the application of controlled and quantified axial mechanical load. Twenty New Zealand skeletally mature white rabbits weighing an average of 3.6kg (conducted under a protocol approved by Institutional Animal Care and Usage Committee, National University of Singapore, Singapore) were randomly assigned into four groups equally. Under general anaesthesia, the surgery was performed through a dorsal approach to the lumbar spine. The custom-made external loading device was attached using four stainless steel pins placed percutaneously and attached to two K-wires inserted into the vertebral bodies L4 and L5 parallel to the adjacent study disc. After the wound was closed, in 15 animals (Groups A, B, and C), axial stress to the disc was created to produce a disc compressive force of 177N for 28 days, equivalent to 5 times the animal’s body weight. After 28 days, group B proceeds with unloading for 28 days. In group C, the compression device was removed and the distraction device was externally attached to the vertical pins. Distraction load was applied by a flexible spring mechanism for another 28 days. Group D rabbits underwent a sham operation with external loading device attached but without any compression or distraction. At the end of the study, the animals were euthanized, and the lumbar segments harvested for analysis.

Micro CT images were taken to examine their disc height and architecture of both the cartilage and the ossaneous components of the endplate. Disc height was calculated according to W. Frobin et al. Hematoxylin-eosin (H&E) staining method was used to examine the general morphologic features of the endplate and the intervertebral disc. Disc histology were analyzed and scored using a modified scoring system proposed by Masuda et al and Boos et al. Disc height ratio and histological scoring were statistically analyzed by comparative test of 2 samples (t test) between any two groups, and one way ANOVA for the difference between the 4 groups. A 2-tailed P value ≤0.05 was considered significant. Data analysis was performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego CA).

RESULTS:

Comparing the 4 groups, group A had the lowest disc height ratio (0.923 ± 0.099) while group C had the highest disc height ratio (1.282 ± 0.322). Group C had significant increase of disc height, as compared to group A (P=0.037). In group B (0.984 ± 0.097) after unloading, the disc height did not change significantly, when compared to group A (P=0.037). Group C had significant increase of disc height, as compared to group A (P=0.037). Group C had significant increase of disc height, as compared to group A (P=0.037). Group C had significant increase of disc height, as compared to group A (P=0.037). Group C had significant increase of disc height, as compared to group A (P=0.037). Group C had significant increase of disc height, as compared to group A (P=0.037). Group C had significant increase of disc height, as compared to group A (P=0.037). Group C had significant increase of disc height, as compared to group A (P=0.037).

In microCT features of vertebral endplate, the cartilaginous endplate was seen as the gap between the osseous endplate and the vertebral body. Partial calcification of the cartilaginous endplate was apparent in the caudal endplate of groups A, B and C but none in group D. The partial calcification was denoted by the narrowing of the gap or the merger of both the vertebral bodies and the osseous endplate surface (Figure 1).

Figure 1. Left column: Intervertebral disc (H&E stained); Middle column: Nucleus pulposus cells (H & E stained); Right column: Midsagittal view of intervertebral disc (2D microCT images)

DISCUSSION:

Our study used µCT imaging and histological assessment to reveal a relationship between the intervertebral disc and endplate in a rabbit model. The µCT imaging showed partial ossification of the cartilaginous endplate after compression but none in the sham operated group. Based on the observations, degeneration of the cartilaginous endplate might play a role in intervertebral disc degeneration. Our observation indicated that bony sclerosis was accompanied by degeneration of the intervertebral disc. Ossification of the endplate was accompanied by condensation of the extracellular matrix in the nucleus pulposus, which implied a decrease in proteoglycan aggrecans. Calcification of the cartilage resulted in the decrease in proteoglycan aggrecans, and the depletion of proteoglycans from the cartilaginous endplate was associated with loss of proteoglycans from the nucleus.

Proteoglycan molecules within the matrix are critical for the control of solute transport and especially maintenance of water content throughout the disc. Condensation of the extracellular matrix in our study implied that there was a decrease in water content which implied a decrease of proteoglycan molecules, and hence the reduced efficiency to maintain water content. As a result, the loading forces could not be distributed evenly to the annulus fibrosus, and this contributed to the degeneration of the disc. Histology showed the restoration of extracellular matrix in both the endplate and nucleus pulposus upon unloading and distraction. Hydration of the nucleus pulposus was observed and implied the increase in proteoglycan aggrecans in the event of unloading and distraction. This was associated with the regeneration of the intervertebral disc. Further experimental studies are needed to investigate the effect of endplate degeneration on disc health.