Effect of diminished flow in rabbit lumbar arteries on intervertebral disc matrix changes: MRI T2-mapping and histological study

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INTRODUCTION: Clinical and epidemiological studies have shown that atherosclerosis of lumbar arteries, branching arteries of the abdominal aorta, is associated with lumbar disc degeneration and low back pain [1]. The lumbar intervertebral disc (IVD) is the largest avascular tissue in the adult human body. Its nutrition occurs by means of diffusion from blood vessels in the surrounding structures [2, 3]. IVDs are supplied by capillaries that arise in the vertebral bodies and penetrate the subchondral bone. Since the IVDs are located at the end of the blood supply, factors that reduce the blood supply to the lumbar spine may cause micro-environmental changes within a lumbar intervertebral disc. However, the direct effect of impaired flow in lumbar arteries on intervertebral disc matrix changes has not been examined. The purpose of this study was to evaluate IVD matrix changes in a rabbit lumbar artery ligation model.

METHODS: Rabbit lumbar artery ligation model: New Zealand White rabbits (n=20, 12-weeks-old), weighing 2.4-3.0 kg, were used in this study with institutional animal care committee approval. Under general anesthesia, the abdominal aorta was exposed and the third and fourth lumbar arteries that arise from the aorta were doubly ligated using vascular clips. The blood flow of the L3/L4 disc (the caudal disc) was diminished by ligation of the third lumbar artery (caudal side to the L3/L4 disc), and the L5/L6 disc (the cranial disc) by ligation of the fourth lumbar artery (cranial side to L5/L6 disc), while the L4/L5 disc (the bilateral disc), by the ligation of the third and forth lumbar arteries (both cranial and caudal side to the L4/L5 disc). At 4, 8, 12 weeks after the surgery, five rabbits per group were sacrificed and processed for MRI and histological analyses. Furthermore, five rabbits (12-weeks-old) without surgery were used as 0 week (control) group.

Radiographic Analysis: Disc height was radiographically monitored biweekly from the day of operation to 12 weeks post-operation. IVD height was expressed as the disc height index (DHI). Percent DHI (%DHI = [postoperative DHI - preoperative DHI] x 100) was subsequently calculated [4].

MRI Analysis: MRI examinations were performed on all rabbits in the study using a 3.0-Tesla (Achieva 3.0T, PHILIPS, Amsterdam, The Netherlands). T2-weighted images in the sagittal plane were obtained using standard clinical multi-echo spin T2 mapping sequence. For T2-quantification, the mean signal intensities were determined in the regions of interest (ROD) framing the inner border of AF. The T2-values were normalized to those of L2/L3 disc.

Histological Analysis: After MRI assessment, the experimental IVDs were fixed in 4% formalin, decalcified, embedded in paraffin, sectioned mid sagitally, and stained with either hematoxylin and cosin, or safranin-O. The observer, blinded to the experiment, analyzed the histologic sections and graded them using a previously reported grading scale [4]. In addition, histological changes of the pericellular matrix of nucleus pulposus cells, the cartilaginous end-plate, and the subchondral bone were also evaluated and graded (grading scale: 1-3). The total score of all grading scales of each disc was statistically analyzed.

Statistical Analysis: Differences of %DHI, MRI T2-values and histological grading scores were assessed for statistical significance by two-way repeated measures ANOVA and the Bonferroni’s multiple comparison.

RESULTS: Change in DHI: The disc heights of the caudal, bilateral and cranial discs were slightly decreased after ligation of lumbar arteries. However, there were no significant changes in disc height over the experimental period.

MRI T2 quantification: The intensity of IVDs had changed after the ligation of lumbar arteries compared to the control disc (Fig. 2A). The MRI T2 values of the bilateral disc showed a tendency to decrease until 8 weeks, although the T2 values recovered at 12 weeks (bilateral disc, 4w: 89.2%, 8w: 86.8%, 12w: 91.3%) (Fig. 1). In the caudal and cranial discs, T2-values of the nucleus pulposus tended to decrease at 4 weeks and returned to recovery all weeks (caudal disc, 4w: 93.1%, 8w: 95.1%, 12w: 94.3%; cranial disc, 4w: 85.0%, 8w: 88.0%, 12w: 95.2%) (Fig. 1).

When the effect of ligation of lumbar arteries was analyzed using the 2-way ANOVA, a significant reduction in T2-value was observed at 4 and 8 weeks after the surgery compared to the control (0 week) discs (P<0.01, respectively), although there were no significant differences among the three disc groups at any time point.

Histological Grading: After ligation of lumbar arteries, safranin-O staining properties of corresponding IVDs had changed. At 8 weeks, condensation of the extracellular matrix (intense staining for safranin-O in/around the pericellular) of nucleus pulposus tissue was found (Fig. 2B). The ligation of lumbar arteries significantly affected the histological total score of corresponding IVDs. When the overall differences among the three groups were assessed using 2-way ANOVA, a significant reduction in T2-value was observed at 4, 8 and 12 weeks after the surgery compared to the control discs (0 weeks) (P<0.01, respectively). However, there were no significant differences among the three groups at any time point.

DISCUSSION: We have established a rabbit lumbar artery ligation model and evaluated the matrix changes of the corresponding IVD. Diminished flow in the lumbar arteries affects changes in MRI T2 values and histology grading in the corresponding IVDs. However no significant differences on matrix changes were found among the caudal, bilateral and cranial disc groups. The recovery of T2 values at 12-weeks would be attributed to the development of collateral arteries surrounding the lumbar spine. The results of this study suggest that diminished flow in lumbar arteries can affect changes in the extracellular matrix metabolism of the IVD.

SIGNIFICANCE: This study showed, for the first time, that diminished flow in rabbit lumbar arteries affects extracellular matrix changes of the lumbar IVDs. The results of this study suggest that degenerative lumbar diseases (degenerative disc diseases) could be developed by ischemic changes of the lumbar spine.

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