Effect Of A Selective Inhibitor Of c-Fos/activator Protein-1 On Intervertebral Disc Degeneration Induced By Needle Puncture In Rats

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Introduction: Intervertebral disc (IVD) degeneration, a cause of low back pain, is associated with an imbalance between the production and destruction of extracellular matrix (ECM), especially in the nucleus pulposus (NP). Several catabolic, anabolic and inflammatory factors play important roles in improving this imbalance in ECM. c-Fos/AP-1 is a transcription factor which controls the expression of both inflammatory cytokines and matrix-degrading matrix metalloproteinases (MMPs) by binding directly to AP-1 motifs in the promoter of these genes (1). T-5224 is a newly designed selective inhibitor of c-Fos/AP-1 which has shown efficacy in a mouse model of rheumatoid arthritis (1). Here, we investigated whether T-5224 prevents disc degeneration in a rat needle puncture model (2).

Methods: Reagents
T-5224 (c-Fos/AP-1 inhibitor), 3-(5-[4-(Cyclopentyloxy)-2-hydroxybenzoyl]-2-[(3-hydroxy-1, 2-benzisoxazol-6-yl)methoxy]phenyl)propionic acid was newly synthesized (1) and provided by Toyama Chemical Co., Ltd.
Animals and procedure
A total of 32 12-week-old male Sprague Dawley rats were anesthetized and punctured with a sterile 20-gauge needle from the dorsal to the ventral side of the tail. Two puncture methods were used: Method I, puncture with half-penetration (to a depth of 5mm; approximate perpendicular distance from the skin) (Fig. 1a); and Method II, puncture with full-penetration from the ventral to dorsal skin through the center of the NP (Fig. 1b). Co5/6 was not punctured and used as the control, Co6/7 was punctured by Method I, and Co7/8 was punctured by Method II. After recovery from anesthesia for 24 hours, T-5224 or PVP (polyvinylpyrrolidone; vehicle solution) was given by single daily oral administration. Fourteen rats were euthanized at four weeks from the start of oral administration and eighteen rats at eight weeks.

Fig. 1 Procedure for needle puncture: (a) Method I, half puncture (Co 6/7); and (b) Method II, full puncture (Co 7/8)
Image analysis
X-ray and magnetic resonance imaging (MRI) of the rat tail (Co5/6, 6/7, and 7/8) were performed after euthanasia. Intervertebral disc space on x-ray was evaluated using disc height index (DHI) (3). Coronal T2 mapping images were obtained using a Varian Unity Inova 4.7 T MRI and evaluated for disc degeneration. Regions of interest (ROI) after T2 mapping were defined using Image J as the square area which was considered to represent the NP. After an average T2 value of the ROI at each disc level was calculated, the values of Co6/7 and Co7/8 were recalculated using the Co5/6 (control) value as reference (MRI index). The DHI and MRI index in the PVP and T-5224 groups were compared.

Histologic analysis
After x-ray and MRI, the entire intervertebral disc with a part of both the cranial and caudal vertebrae were dissected and fixed in 10% formalin neutral-buffered saline. Samples were then processed for paraffin embedding, decalcification, and coronal sectioning (10 μm thick) using a microtome. Sections were stained with hematoxylin and eosin (HE) and safranin-O. Disc degeneration was quantified using a histological grading scale as previously described (3). Grading was conducted by three orthopaedic surgeons under a blinded approach. The histological grading score was compared between PVP and T-5224 groups.

Statistical analysis
Student-t test was used to compare between two groups for DHI, MRI index and histological grading score. A p value of less than .05 was considered statistically significant. Also, the inter-observer reliability as an intraclass correlation coefficients (ICC) was calculated for histological grading score.

Results: Image analysis
On x-ray analysis, no significant differences were seen between animals treated with PVP or T-5224 for four weeks with either puncture method (p=0.42, p=0.07). At eight weeks, DHI was significantly higher in the T-5224 group than in the PVP group with both puncture methods (p=0.002, p=0.04, Fig. 2a). On MRI analysis, MRI index was significantly higher in the T-5224 group than in the PVP group except with the full puncture method at four weeks (Fig. 2b). The T2 mapping images showed greater disc degeneration in the PVP than in the T-5224 group (Fig. 2c).
Histologic analysis

There were no significant differences between the PVP and T-5224 groups on treatment for four weeks with the half puncture method. In contrast, histologic grade was significantly higher in the T-5224 than PVP group on treatment for four weeks with the full puncture method \( (p=0.05) \), or with both methods on treatment for eight weeks \( (p=0.02, p=0.04, \text{Fig. 3a}) \). The PVP group displayed a loss of NP tissues compared to T-5224 group in safranin-O staining \( \text{Fig 3b}) \).

Discussion: We showed that a selective c-Fos/AP-1 inhibitor prevented IVD degeneration in a rat needle puncture model. These findings suggest that catabolic and inflammatory factors located in the
downstream of c-fos were suppressed by T-5224. Given that c-fos expression inhibits aggrecan and Col2 promoter activities and the expression of these genes in NP cells (4), these findings suggest that inhabitation of c-fos is likely to be useful in preventing IVD. T-5224 can be administered orally in humans, and its safety has been established. The selective c-Fos/AP-1 inhibitor T-5224 appears suitable for development as a therapeutic agent for the treatment of IVD degeneration in humans.

**Significance:** A selective c-Fos/AP-1 inhibitor prevented disc degeneration in a rat model of IVD degeneration induced by needle puncture, and may have considerable potential as a therapeutic agent for the treatment of IVD degeneration.

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