The Efficacy of Growth Differentiation Factor-6 on Intervertebral Disc Degeneration in the Rabbit Anular-puncture Model

Shingo Miyazaki[1, 4], Kenji Kato[1], Kevin Cheng[1], Won C Bae[2], Yang Sun[3], Weibo Jiang[3], Junichi Yamada[1], Edward Abarado[1], Erikka Linn[1], Carol Muehleman[1], Mary E Lenz[1], Robert L Sah[3], Ashish D Diwan[5], *Koichi Masuda[1]

[1]Departments of Orthopaedic Surgery, [2]Radiology and [3]Bioengineering, University of California, San Diego, La Jolla, CA, [4]Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan, [5]Orthopaedics Research Institute and Department of Orthopaedic Surgery, St. George Hospital University of New South Wales, Sydney, Australia.

Email: shmiyazaki@ucsd.edu *Correspondence; komasuda@ucsd.edu

DISCLOSURES: Miyazaki S, Kato K, Kevin C, Bae WC, Sun Y, Jiang W, Yamada J, Abarado E, Linn E, Muehleman C, Lenz ME, Sah RL, Diwan AD, Masuda K (N)

INTRODUCTION: Degenerative disc disease is a common disorder that causes low back pain and dysfunction. Recent studies revealed that growth factors, such as bone morphogenetic proteins (BMPs), have positive effects on extracellular matrix (ECM) metabolism and cell proliferation *in vitro* and induce structural repair of intervertebral discs (IVDs) in various animal models (1). Growth differentiation factor-6 (GDF6/BMP13), known to play an essential role in skeletal development (2), is expressed by both human degenerative and scoliotic IVDs. GDF6 increased the expressions of Collagen-I (Col-I) and Col-II and proteoglycan accumulation by human nucleus pulposus (NP) and endplate cells (3). A single injection of GDF6 at the time of anular stab in sheep has shown positive effects on preserving disc structures (X). To reveal clinical efficacy, in this study the effects of a single injection of various doses of GDF6 on disc height (2D and 3D) and qualitative and quantitative MRI assessment were determined in the rabbit anular-puncture model.

METHODS: The study protocol was approved by the Institutional Animal Care and Use Committee.
Rabbit anular-puncture disc degeneration model and the injection of GDF6: Female New Zealand white rabbits (n=32) were used in this study. Under general anesthesia, lumbar IVDs were exposed and the anulus fibrosis was punctured with an 18-gauge needle (5 mm depth) in two non-contiguous discs (L2/3 and L4/5). Four weeks after the initial puncture, either vehicle (phosphate-buffered saline (PBS); 10 µL per disc) or growth factor GDF6 (1, 10 or 100 µg in PBS, 10 µL per disc; PEPROTECH, Rochey Hill, NJ, USA) was injected into the center of the NP using a 26-gauge needle.
Radiographic analysis of disc height: Lateral radiographs of the lumbar spine were obtained every two weeks. IVD height was expressed as disc height index (DHI) and normalized to L3/4. DHI were normalized to the preoperative DHI (%DHI) and further normalized to the DHI of the L3/4 non-punctured disc.
Micro CT and three-dimensional (3D) disc height distribution (DHD [whole disc and zonal]): The spine segments were imaged using the Skyscan 1076 µCT scanner (Kontich, Belgium). Using Mimics (Materialize, Plymouth, MI, USA) 3D surfaces were reconstructed. The average minimum distance between apposing bony endplates was calculated as the DHD and further normalized to the DHD of L3/4. To determine regional repair variations, DHDs in five zones (posterior, left-lateral, anterior, right-lateral, and central regions) were computed.
MRI analyses: A fast spin-echo T2-weighted sequence was performed using 7-Tesla MRI. The degeneration grade of IVDs was classified according to Pfirrman grading using T2 weighted images (TE=64 ms). For T2-quantification, masks of the L3/4 NP (template for normal NP) were applied to L2/3 and L4/5 discs by affine registration of the template to match the AF of the target degenerated discs. The registered ROI was applied to T2 maps to determine average T2 values of the NP and AF of each disc. All T2 values were normalized to the values of the L3/4 non-punctured control disc.
Histological analyses: Midsagittal histologic sections (HE and Safranin O) were graded using our established protocol [4]. Grades ranged from 4 to 12, where normal is 1 point for each of the 4 categories, for a total of 4 points (total 12 points).
Statistical analyses: All data are expressed as mean and standard error (SE). Comparisons among the experimental groups were analyzed using two-way or two-way repeated analysis of variance (ANOVA, DHI, DHD, MRI quantification, histological grade) or the Kruskal-Wallis test (MRI degeneration grade).

RESULTS: DHI ***(Fig. 1A)***: Repeated two-way ANOVA revealed that treatment significantly affected %DHI in the GDF6 10 and 100 ug groups (GDF6 1 µg; P=0.17, 10 µg; P<0.05, 100 µg; P<0.01 vs. PBS group). At week 16, three GDF6 groups showed a significant increase in %DHI.
DHD ***(Fig. 1B, C)***: There were no significant differences among the groups in the average normalized DHD. However, the zonal DHD of the posterior region in the GDF6 groups was significantly higher than that in the PBS group (GDF6 1 µg; P<0.05, 10 µg; P<0.01, 100 µg; P<0.01 vs. PBS group).
MRI degeneration grade ***(Fig. 2A, B)***: The Pfirrman grade was significantly lower (less degeneration) in the GDF6 10 and 100 µg groups compared to the PBS group (GDF6 1 µg; P=0.75, 10 µg; P<0.05, 100 µg; P<0.05 vs. PBS group).
Quantitative MRI assessment: T2 values of both the NP and AF in the GDF6 groups did not show significant differences compared to those in the PBS group (data not shown).
Histological grade: GDF6 injection did not show any significant effects compared to PBS injection (data not shown).

DISCUSSION: The injection of GDF6 into the NP at four weeks after anular puncture restored disc height and the zonal analysis of disc height revealed that the restoration was significant in the posterior zone. MRI qualitative assessment also indicated positive effects of GDF6 injection at 10 and 100 µg/discs on disc hydration. No apparent acceleration of bone formation was found in micro CT analysis. A separate study indicated that GDF6 injection at 100 µg induced inhibition of cytokines at four weeks after the injection; this supports our contention that GDF6 shifts the metabolic status to anabolism, thus inducing structural repair and reducing the progression of disc degeneration. The delay in disc height recovery (4-6 weeks post-injection) may be due to a delayed biological response to the injection. Metabolic studies of proteoglycan and collagen synthesis at these time points may shed light on the mechanism of disc repair.

In conclusion, an injection of GDF6 may change the pathological status of degenerated discs and serve as a new therapeutic approach for degenerative disc disease.

SIGNIFICANCE: The administration of GDF6 has the potential to repair degenerative IVDs.

REFERENCES: (1) Masuda K+, Spine. 2006;31:742-54; (2) Shen B et al., Int J Bio Sci. 2009;5:192-200; (3) Gulati T et al., J Orthop Res. 2015;33:1769-75; (4) Masuda K+, Spine. 2005;30:5-14.

FIGURES:

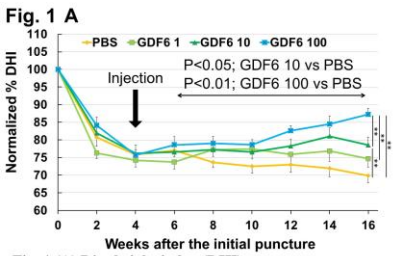
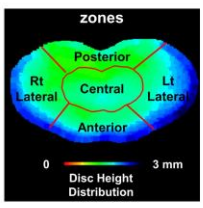
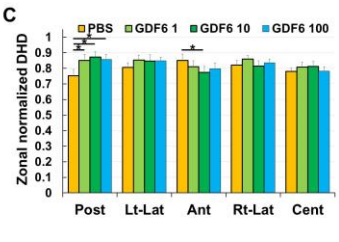


Fig. 1 (A) Disc height index (DHI)



(B) Zones of disc height distribution (DHD)



(C) Normalized zonal DHD

Data are expressed as mean and standard error (SE). *P< 0.05; **P< 0.01

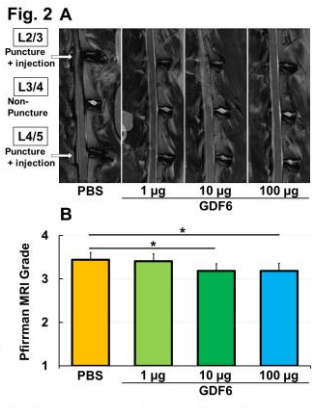


Fig. 2 (A) Representative cases (MRI T2 sagittal view)
(B) Pfirrman disc degeneration grade