INJECTION OF OSTEOGENIC PROTEIN-1 INDUCES STRUCTURAL RESTORATION OF A DEGENERATED RABBIT INTERVERTEBRAL DISC IN AN ANNUAL PUNCTURE MODEL

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INTRODUCTION: Growth factors, such as osteogenic protein-1 (OP-1), have the ability to stimulate synthesis of proteoglycans (PGs) and collagen by intervertebral disc (IVD) cells in vitro [1]. A recent study showed that the injection of OP-1 into normal rabbit IVDs induced an increase in disc height. The co-injection of OP-1 and chondroitinase ABC, a chemonucleolytic agent, into the rabbit IVD resulted in a significant recovery of disc height following initial chemonucleolytic effects [2]. In addition, a single injection of growth differentiation factor-5 into degenerated mouse caudal discs, induced by static compression, resulted in a significant effect on disc height loss [3]. These results suggest that an injection of a growth factor may have therapeutic use in the biological repair of degenerated IVDs by stimulating anabolic activities by these cells. To test this therapeutic concept, we conducted an in vivo study that encompassed injecting OP-1 into the nucleus pulposus (NP) of a rabbit IVD undergoing degeneration following needle puncture as recently described [4].

The specific purpose of this study was to determine the effects of intradiscally-administered OP-1 on the progression of disc degeneration in the rabbit IVD degeneration model by monitoring radiographic changes and MRI findings in vivo, as well as histological and biochemical changes.

METHODS: The needle puncture model and OP-1 injection: Seventy-two adolescent New Zealand white rabbits (weighing 3.5-4 kg) were used in this study with institutional animal care committee approval. Under general anesthesia, the annulus in two non-contiguous discs (L2/3 and L4/5) was punctured with an 18G needle using the left retroperitoneal approach, to induce disc degeneration. Four weeks later, eight rabbits per group were sacrificed for baseline assessments of the annular puncture; the remaining rabbits were assigned to two treatment groups. One group received an injection of 5% lactose (10 µl) and the other group received an injection of OP-1 (100 µg in 10 µl of 5% lactose) into the NP of the two punctured discs using a contralateral approach. The disc (L3/4) between the punctured discs was used as a non-punctured control disc.

Radiological and MRI Assessments: Disc height was radiographically monitored biweekly from the day of needle puncture injury to 24 weeks post-injection. IVD height was expressed as the disc height index (DHI) [2]. Percent DHI (%DHI = postoperative DHI/preoperative DHI) x100 was subsequently calculated. At 4-, 8-, 12- and 24-weeks after injection, eight rabbits per group were sacrificed and an MRI of the spinal column was taken to grade the level of degeneration based on modified Thompson grade (MRI, 1=normal, 4=severely degenerated) [4].

Histological Analysis: The punctured disc from one level was used for histological analysis and the other punctured disc and control disc were used for biochemical analyses (DNA, PG and collagen contents). The histological sections were analyzed and graded on four parameters using an established grading scale with grades ranging from a normal score of 4 to a severely degenerated score of 12 [4].

Biochemical Analysis: At each time point, the discs were dissected and the NP and annulus fibrosus (AF) separated. All specimens were digested with papain at 60°C for 24 hours. The DNA content was determined by the Hoechst 33258 dye method [1]. The PG content was assayed by the DMMB assay and hydroxyproline, as a measure of collagen, was quantified by reverse-phase HPLC [1].

Statistical Analysis: The significance of differences of the means between the two groups was assessed using two-way ANOVA and Fisher’s LSD as a post hoc test.

RESULTS: Change in DHI: The annulus needle puncture resulted in consistent disc-narrowing within four weeks (a 29% decrease). The injection of OP-1 induced a significant restoration of disc height 6 weeks after the injection of OP-1 (i.e. 8-weeks post IVD puncture). At 8-weeks, OP-1 induced restoration of disc height, to a level approaching that of non-punctured controls; this was sustained for the entire experimental period up to 24-weeks after the injection of OP-1 (Fig. 1). The injection of lactose did not change the course of disc narrowing over the same time period. MRI Grading: MRI grading at each time point showed a significant difference between the control and OP-1 groups at the 8-, 12- and 24-week time points, suggesting an increase of water content in the NP (8w: p<0.01; 12w: p<0.01; 24w: p<0.05) (Fig. 2). Histological Grading: Histologically, the degeneration grades of the punctured discs in the OP-1 group were significantly lower than those in the lactose group (Mann-Whitney, p<0.05) (Fig. 2). Biochemical Analyses: The needle puncture injury induced dramatic decreases in DNA and PG content in the NP during the first four weeks (DNA: 24%; PG, 29% of non-punctured control) while the AF showed only slight decreases (DNA: 84%; PG, 89% of non-punctured control). The injection of OP-1 induced a significant increase in the PG content (p<0.01) in the NP and AF, but the increases in DNA and collagen contents did not reach statistical significance.

DISCUSSION: In the rabbit annular puncture disc degeneration model, a single injection of OP-1 induced an improvement in MRI grading score and the recovery of disc height. The biochemical and histological results support the results of the radiographical and MRI studies. The PG content in the NP and AF of OP-1-injected IVDs was significantly elevated compared to that of the lactose group; it was higher than that of the non-punctured control group in the AF. Importantly, the effect of the OP-1 injection was sustained for 6 months after injection. The results of this study demonstrate the feasibility of restoring degenerative discs in the rabbit by a single injection of OP-1. Because the phenotype of rabbit NP cells differs from that of human NP cells, an efficacy study in large animals is required to prove that the intradiscal injection of OP-1, or a similar growth factor, is useful for the biological restoration of the IVD in the human.


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