INTRODUCTION: Changes in the biomechanical properties of degenerated intervertebral discs (IVDs) are associated with biochemical changes in the extracellular matrix (ECM) of the IVD, such as loss of proteoglycan (PG) and collagen, which lead to subsequent instability and inflammation. Recently, as a minimally invasive therapeutic approach for degenerated IVDs, biological repair using growth factors has gained interest.

Osteogenic protein-1 (OP-1) is known for its efficacy in stimulating ECM production and restoring disc height in the rabbit disc degeneration model [1,2]. However, whether these effects of OP-1 result in restoration of the biomechanical properties of IVDs has not been explored. The present study was performed to investigate the effects of an intradiscal injection of OP-1 on both biomechanical and biochemical properties using the rabbit IVD annular puncture model [3] and a newly developed dynamic viscoelastic property testing system [4] to reveal any relationship between these two properties.

METHODS: Operations: Fourteen New Zealand White rabbits (3-3.5 kg) underwent annulus fibrosus (AF) puncture (18G) at both the L2/3 and L4/5 discs to induce disc degeneration [3]. The L3/4 discs served as non-punctured controls. Four weeks later, both punctured discs in each animal received the same injection of either 5% lactose (10 µl) or OP-1 (100 µg/10 µl) into the nucleus pulposus (NP). Eight weeks after the injection, lumbar spines were harvested after sacrifice and bone-disc-bone complexes were removed.

Dynamic Viscoelastic Properties: When sinusoidal strain is applied to viscoelastic materials, the resultant stress will be out-of-phase with a time-lag called phase angle δ. A complex modulus K* has two components: the elastic modulus K’ and the viscous modulus K”. K’ = K’ + iK”, where i = √-1. [5,6] The magnitude is determined by |K*| = ε(stress)/ω(length change)/DH(disc height). Finally, the properties are defined as K’ = |K’| cos δ, and K” = |K’| sin δ [5].

Testing Protocol: Using a custom-made biomechanical testing system [4], a cycle of sinusoidal strain, ε(t) = -εo + εo cos(ωt), was applied to each IVD in an uniaxial unconfined compression. Strain amplitude of, εo = 10%, 10% disc height was applied. The average resultant force of this study was 95 N, which represents 249% of the rabbit’s body weight. After pre-conditioning by 10 cycles at 1 Hz, six different physiological loading frequency tests (0.05, 0.1, 0.2, 0.5, 1, and 2 Hz) were performed. Radiological Analysis: Disc height was radiographically monitored biweekly. Disc height was expressed as the disc height index [%DHI = (Post-operative DHI/Pre-operative DHI)x100] [3].

Biochemical Analyses: After the biomechanical testing, the NP and AF were separated. Specimens were analyzed for DNA content using the Hoechst 33258 dye method, PG content by the DMMB method [1]. Correlations between PG content and dynamic viscoelastic parameters (K’ and K”) were examined using the Spearman’s signed rank test.

Statistical Analysis: Repeated ANOVA or one-way ANOVA with Fisher LSD test was used.

RESULTS: Disc Height: Annulus puncture induced a 32% decrease in disc height four weeks after puncture (Fig. 1). At 4-weeks post-injection, OP-1 injections significantly restored disc height; this was sustained for up to eight weeks after the injection (OP-1 vs Lactose, p<0.001, Fig. 1).

Biomechanical Properties: The elastic modulus (K’) in the lactose group (triangle) was significantly lower than that in the control group (closed circle) at all loading frequencies (mean: 55%, p<0.001). At all loading frequencies, K’ in the OP-1 group was significantly higher than that in the lactose group (mean: 41%, p<0.001) and approached that of the control level (OP-1 vs Control, n.s.) (Fig. 2, left). The viscous modulus (K”) in the OP-1 group was significantly higher than that in the lactose group at 0.05, 0.2, and 1 Hz (average: 56%, p<0.001) while showing a strong tendency to be higher at 0.1, 0.5, and 2 Hz (p = 0.06-0.10) (Fig. 2, right). In both the elastic and viscous moduli, no significant difference was observed between the OP-1 and control groups at all loading frequencies.

Biochemical Properties: The DNA contents of both the NP and AF in the OP-1 group were slightly but significantly higher than those in the lactose group (p<0.001). The PG contents of both the NP and AF in the OP-1 group were significantly higher than that in the lactose group (Fig. 3). The elastic modulus (K’) showed a positive correlation with total PG content in the NP (p<0.05) (Fig. 4).

DISCUSSION: We have shown for the first time that an injection of a growth factor, OP-1, successfully restored the disc height and biomechanical properties of the IVD in the rabbit IVD annular puncture model. The results of the positive correlation between PG content in the NP and the elastic modulus of the IVD suggest that biochemical changes induced by an injection of OP-1 may result in structural and mechanical restoration. Our data support the feasibility that a single injection of OP-1 may serve as a therapeutic solution for a degenerative IVD to regenerate into “a functioning shock absorber” in well-selected indications. However, because the biomechanical function of human IVDs includes multidirectional flexibility and complex motion, further biomechanical testing of biologically-treated IVDs should be performed in larger animals that more closely resemble the human IVD structure.


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