**INTRODUCTION:** Bone morphogenetic protein-2 (BMP-2), a member of the TGFβ superfamily of proteins, is involved in the cascade of cellular events in bone formation, regeneration and proliferation. BMP-2 is also considered to be an anabolic factor that influences the development and growth of other musculoskeletal tissues; it has been shown to stimulate proteoglycan synthesis by intervertebral disc (IVD) cells in animals and human (1, 2). Several members of the BMP family have been shown to be effective in repairing degenerated discs in animal models (3). However, the *in vivo* effects of BMP-2 are still unknown (4).

**METHODS:** The rabbit anular puncture model and rhBMP-2 injection: Twelve adolescent New Zealand white rabbits (3.5–4 kg) were used with IACUC approval. To induce disc degeneration, the anulus in noncontiguous discs (L2/3 or L4/5) was punctured with an 18G needle, using a left retroperitoneal approach under general anesthesia. Four weeks later, the rabbits received an injection of phosphate buffered saline (10 µl, PBS group, 6 rabbits, 12 discs) or rhBMP-2 (100 µg, in 10 µl PBS, 6 rabbits, 12 discs) into the nucleus pulposus (NP) of previously punctured discs under direct observation. Rabbits were radiographically monitored for 12 weeks after the injection and then sacrificed. The AF and NP were separately dissected, stored in RNA later (Qiagen) solution and pulverized after freezing in liquid nitrogen.

**Radiological Assessments:** Disc height was radiographically monitored biweekly. The percent disc height index [%DHI = (postoperative DHI/preoperative DHI) x100] was calculated as previously described (4). Normalized %DHI was calculated to account for changes in disc height associated with anesthesia during x-ray.

**Quantitative PCR (q-PCR):** Total RNA was isolated from AF and NP tissues and q-PCR was performed using gene-specific primers for IL-1β, IL-6, TNF-α, matrix metalloproteinase-3 (MMP-3), ADAMTS4, ADAMTS5, nerve growth factor (NGF), and cyclooxygenase-2 (COX2). Standards for each were made using the cloning pDrive vector from the PCR cloning kit (Qiagen, CA). GAPDH was used as internal control.

**Statistical Analyses:** Differences between groups were assessed with two-way ANOVA with The Fisher LSD test or Mann-Whitney test.

**RESULTS:** Changes in DHI (Fig. 1): The anulus needle puncture resulted in consistent disc narrowing within four weeks (22% decrease from baseline). After injection, BMP-2 significantly affected disc height (PBS vs. rhBMP-2, p<0.01). At 4 weeks after the injections, the disc height in the BMP-2 group had increased towards that of the pre-puncture level and continued to be significantly higher than that of the PBS group (p<0.01–0.05) 8W–16W after puncture.

**Proinflammatory Cytokine Expression (Fig. 2, A–F):** Comparisons between the two treatment groups revealed that rhBMP-2 significantly suppressed the expression of IL-1β, IL-6 and TNF-α (p<0.01) at 12 weeks after the puncture in both AF and NP tissues.

**Catabolic Enzyme Expression (Fig. 2, G–J):** In the AF and NP, levels of ADAMTS4 were significantly decreased in the BMP-2 group compared to the PBS group (p<0.01). MMP-3 levels of the BMP-2 group were significantly lower than that of the PBS group in the NP (p<0.01).

**Expression of Pain-related Molecules (Fig. 3, K–N):** Significant increases of NGF and COX2 expression were observed in punctured discs (NGF, p<0.05, COX2, p<0.01, vs. PBS). The expression levels of pain-related molecules, NGF and COX2, in both tissues were significantly decreased (p<0.001) in comparison to the PBS group.

**CONCLUSIONS:** The study showed that a single injection of rhBMP-2 induced disc height recovery and significantly decreased the expression of cytokines (IL-1, IL-6 and TNF-α) and ADAMTS4 as well as NGF and COX2 mRNA in the rabbit anular puncture model. Notably, the analyses of mRNA has proven to be useful in detecting metabolic changes occurring with anular puncture and BMP-2 injection. The decrease in the expression of the pain-related molecules, NGF and COX2, suggests that an intraarticular injection of BMP-2 may exert favorable effects on pain alleviation, as well as structural modification of degenerated discs. Additional detailed studies using different doses of BMP-2 will provide more precise insight into the mechanism of the action of BMP-2 on disc degeneration.

**Fig. 1:** Changes of Normalized %DHI after BMP-2 Injection

**Fig. 2:** Gene Expression of Proinflammatory Cytokines, Catabolic Enzymes and Pain-related Molecules in AF and NP tissues


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