BMP-7 is more effective than BMP-2 in reversing the catabolic effect of TNF-α on human disc nucleus pulposus (NP) cells.

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INTRODUCTION:
BMP-2 and BMP-7 are somewhat similar bone morphogenetic proteins. They are both under investigation for possible use in treating disc degeneration and low back pain. Both molecules can enhance disc cell synthesis of chondrogenic molecules in standard in vitro culture conditions without inflammatory cytokines. In vivo however, disc degeneration is more complex and seems to include inflammatory processes involving tumor necrosis factor-α and NF-kappa-B (NFKB) pathway signaling. The aim of this study was to investigate whether there are any differences in the ability of BMP-2 and BMP-7 to reverse the effect of TNF-α on disc matrix synthesis in human intervertebral disc nucleus pulposus (NP) cells.

METHODS:
Cell preparation: Human nucleus pulposus (NP) tissues were obtained from eight patients during surgical procedures carried out by one author (a surgeon) in a university hospital. The cells from the NP tissue were grown in DMEM/F12 with 10% FBS. When the cells reached 80% confluence, they were treated for 2 days in two experiments with: 1) TNF-α (at doses of 0, 5, 10, and 20 ng/ml); 2) TNF-α (at 20 ng/ml) in the presence or absence of 100 ng/ml of BMP-2 or 100 ng/ml of BMP-7. The cells were incubated in DMEM/F12 with 1% FBS.

Real-time PCR: The expression levels of aggrecan and collagen II were measured by real-time polymerase chain reaction. The results are expressed as a ratio to the untreated control group.

Western blot: Cells were homogenized in a PhosphoSafe extraction buffer (EMD Biosciences) containing a protease inhibitor cocktail and 1 µM phenylmethylsulfonyl fluoride. Protein concentrations were determined by Pierce BCA assay. Equal amounts of cellular lysate protein were run on SDS-PAGE, electro blotted onto PVDF membranes, incubated with primary antibodies, and then HRP-conjugated secondary antibodies. The enhanced chemiluminescence detection system (NEN™ Life Science Products, Inc.) was used to detect bound antibodies.

RESULTS SECTION:
Figure 1: Real time PCR showed that TNF-α (at 0, 5, 10, and 20 ng/ml) represses mRNA expression of aggrecan and collagen type II in human disc NP cells in a dose-dependent manner.

Figure 2: Western blots show that TNF-α (at 20 ng/ml) represses expression of aggrecan and collagen II, and increases expression of the NFKB transcription factors p50 and p65 and the catabolic proteolytic enzyme MMP13 in the NP cells.

Figure 3: The NP cells were treated for 2 days with 100 ng/ml of BMP-7 in the presence or absence 20 ng/ml TNF-α. Real time PCR shows that BMP-7 is more effective than BMP-2 in inhibiting TNF-α mediated degradation of aggrecan and collagen II mRNA in the NP cells.

Figure 4: The NP cells were treated for 2 days with 100 ng/ml of BMP-7 in the presence or absence 20 ng/ml TNF-α. Western blots show that BMP-7 represses expression of NFKB transcription factors p50 and p65 and MMP-13 in TNF-α treated NP cells.

DISCUSSION:
Our major findings in relation to human NP cells are as follows: (1) TNF-α represses mRNA expression of aggrecan and collagen type II; (2) TNF-α treatment induces degradation of aggrecan and collagen II and an associated upregulation of NFKB transcription factor and MMP-13; (3) BMP-7 was better than BMP-2 in reversing the TNF-α induced degradation of aggrecan and collagen II. This was associated with down-regulation of NFKB transcription factors p50 and p65 and MMP-13. Our study indicates that BMP-7 is more effective than BMP-2 in reversing the catabolic effect of TNF-α. Our study also suggests that NFKB transcription factor may involved in this process.

REFERENCES: