BONE MORPHOGENETIC PROTEIN PROTECT HUMAN INTERVERTEBRAL DISC CELLS IN VITRO FROM APOPTOSIS

Orthopedic Research Institute, St George Hospital Campus, University of New South Wales, Sydney, NSW, Australia
da.diw@spine-service.org

Introduction
Disc degeneration includes dysfunction and loss of disc cells leading to a decrease in extracellular matrix components (1). Apoptosis has been identified in degenerated discs. Bone morphogenetic protein-7 (BMP-7) is known to stimulate both cellular proliferation and extracellular matrix synthesis in the intervertebral disc but its protective role in apoptosis is unknown. Single dose local BMP-7 can reverse chemically induced disc degeneration in a sheep model (2). The aim of this study was to determine whether BMP-7 protects cultured intervertebral disc cells following stimulation of apoptosis.

Materials and Methods
Nucleus pulposus tissues were obtained from consented individuals undergoing surgical procedures on the disc and digested with collagenase prior to culturing. Cellular apoptosis was achieved by either tumor necrosis factor-alpha (TNF-α) or hydrogen peroxide (H2O2) incubation. Recombinant human BMP-7 (rhBMP-7, Stryker) was used at 100ng/ml, 5 hours prior to the addition of apoptotic stimulation. Cellular apoptosis was detected by TUNEL assay, caspase-3 activity and caspase-3 protein expression. Cellular proliferation and viability was assayed by [3H]-thymidine incorporation and MTS assay respectively. Collagen II and aggrecan protein levels were estimated using western blots and immunostaining. Proteoglycan synthesis was determined by (35)S-sulfate incorporation method. Nitric oxide and alkaline phosphatase activity were measured.

Results
Apoptosis were induced in the nuclear cells by TNF-α or hydrogen peroxide (H2O2) with increased proteolytic activity of caspase-3 as well as cellular shrinkage and nuclear condensation. Addition of BMP-7 prior to stimulation of apoptosis resulted in complete inhibition of the apoptotic effects of both inducers (Fig.1 & 2), as well as the cellular nitric oxide induced by TNF-α (not shown). In the presence of rBMP-7, extracellular matrix (ECM) production was maintained by the cells despite being in an apoptotic environment (Fig.3 & 4). BMP-7 increases cellular viability (not shown). No osteoblastic induction of the disc cells was seen (Fig.5).

Discussion and conclusion
BMP-7 prevents apoptosis of cultured human disc cells induced by either tumor necrosis factor-alpha (TNF-α) or hydrogen peroxide. Induction of apoptosis led to down regulation of extracellular matrix proteins, decreased cell viability, morphological changes and activation of caspase-3, however addition of BMP-7 alone prevented the effects observed. One possible mechanism of the anti-apoptotic effects of BMP-7 was shown by its retardation of the elevated levels of TNF-α induced nitric oxide. Our study offers a molecular rationale for the potential use of rhBMP-7 for disc degeneration.

Affiliated institutions for co-authors
**Department of Orthopaedic Surgery, Sahlgrenska University Hospital, Gothenburg, Sweden

References:

Figure 1. Apoptotic cells shown by TUNEL staining. Brown stained apoptotic cells dominate in TNF-α or H2O2 treated cell cultures. In BMP-7 pre-treated cells, this damage is highly reduced shown by the reduction in apoptotic cells. (Bar=10µm)

Figure 2. (A) Effect of BMP-7 on Caspase-3 activity post-apoptotic induction (N=4, ±SE, P< 0.05). (B) BMP-7 inhibits activated caspase-3 protein expression in cultured disc cells resulting from Western blot.

Figure 3. Effect of BMP-7 on proteoglycan (P.G) synthesis of the NP cells after stimulated with TNF-α measured by [35S]-incorporation assay. (N=4, ±SEM, P< 0.05)

Figure 4. BMP-7 increases extracellular matrix production in an apoptotic environment resulting from Western blot. (A). Collagen-II protein expression following TNF-α or H2O2 treatment and BMP-7 pre-treatment prior to apoptotic induction. (B). Densitometry analysis of the western blot.

Figure 5. Discal cells treated with BMP-7 are absent in bone cellular activity. Alkaline phosphatase activity of cells treated with BMP-7 for 24 – 120 hours compared with that of a positive osteoblastic control