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Technical Review

Bone morphogenetic protein-7 protects human intervertebral disc cells in vitro from apoptosis

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Abstract

BACKGROUND CONTEXT: Disc degeneration includes dysfunction and loss of disc cells leading to a decrease in extracellular matrix (ECM) components. Apoptosis has been identified in degenerated discs. Bone morphogenetic protein-7 (BMP-7) has been reported to stimulate ECM synthesis in the intervertebral disc (IVD), but its effect on disc cell viability is unknown.

PURPOSE: To investigate whether BMP-7 can protect disc cells from programmed cell death while enhancing ECM production.

STUDY DESIGN: An in vitro study to examine the effect of BMP-7 on apoptosis of IVD cells.

METHODS: Human nucleus pulposus (NP) cells were cultured in monolayer, and human recombinant pure BMP-7 (rhBMP-7) was added to the medium when the cells were in the second passage. Thereafter, apoptosis was induced by either tumor necrosis factor-alpha (TNF-α) or hydrogen peroxide (H_2O_2). Cellular apoptosis was evaluated by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay and caspase-3 activity. ECM synthesis was assessed by immunofluorescence for collagen-2 and aggrecan. To study the possibility of bone induction by rhBMP-7 in disc cells, alkaline phosphatase activity and Alizarin red-S staining were evaluated.

RESULTS: Apoptosis was induced by both TNF-α and H_2O_2. Addition of rhBMP-7 resulted in inhibition of the apoptotic effects caused by both inducers. Further, BMP-7 decreased caspase-3 activity. In the presence of BMP-7, ECM production was maintained by the cells despite being in an apoptotic environment. No osteoblastic induction of the disc cells was seen.

CONCLUSIONS: BMP-7 was demonstrated to prevent apoptosis of human disc cells in vitro. One of the antiapoptotic effects of BMP-7 on NP cells might be a result of its inactivation of caspase-3. Collagen production was maintained by addition of rhBMP-7 in an apoptotic environment.

Key words: Bone morphogenetic protein; Tumor necrosis factor; Apoptosis; Intervertebral disc; Disc degeneration; Cell culture

Introduction

The intervertebral disc (IVD) in the spinal column consists of two regions. The outer annulus fibrosus region is composed of parallel layers of collagen-1 and provides the tensile strength to the disc. The inner nucleus pulposus (NP) region of the disc is composed of collagen-2 and proteoglycan (PG), responsible for the retention of water, and provides the viscoelastic properties of the disc. Degeneration of the IVD, associated with chronic low back pain, is an age-, wear-, and injury-related condition characterized by a loss of both extracellular matrix (ECM) and the cells responsible for its maintenance [1–3]. These events may lead to the overall collapse of the disc, contributing to local spinal instability and pain.

This study was conducted after approval from the South-Eastern Health Research Ethics Committee (HREC), New South Wales, Australia.

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Apoptosis or programmed cell death has been shown to be a key component responsible for the decrease in cell number in the NP during degeneration [4–6]. Apoptosis is an important phenomenon involved in normal tissue development and regulation, providing some protection from the onset of malignant transformation of cells. However, if expressed excessively, apoptosis may lead to tissue degeneration [7]. The apoptotic process can be triggered by either an intrinsic (mitochondrial-dependent) or extrinsic (mitochondrial-independent) apoptotic signaling pathway, respectively [8]. Tumor necrosis factor belongs to a family of cytokines including tumor necrosis factor-alpha (TNF-α), Fas-ligand, and TRAIL, which stimulate the extrinsic pathway leading to apoptosis. The intrinsic pathway is instigated by stimuli such as oxidative stress mediated by hydrogen peroxide (H_2O_2) [9]. Both apoptotic pathways have been detected to occur in IVD degeneration, with static mechanical loading–induced degeneration being mediated by the intrinsic pathway and disc herniations being induced by the extrinsic pathway [10–13]. To date two antiapoptotic agents, insulin-like growth factor-1 and platelet-derived growth factor, that retard and prevent in vitro serum starvation–induced apoptosis of discal cells have been identified [14].

Bone morphogenetic protein-7 (BMP-7), a member of the transforming growth factor-β superfamily, is involved in proliferation, differentiation, metabolism, and apoptosis in a variety of tissues [15]. In disc degeneration, BMP-7 has enhancing effects on ECM (PG) synthesis both in vitro and in vivo, with a resultant increase in disc height observed [16–18]. Further, a single dose of BMP-7 has been shown to improve the disc height of degenerated IVD in a sheep model [19]. It is not known how BMP-7 brings about the reversal of the degenerative changes in the IVD.

Apoptotic regulation by BMP-7 is tissue specific, shown by the induction of apoptosis in primary myeloma cells but the rescue of apoptosis in renal cells [20–22]. However, the apoptotic effect of BMP-7 in disc degeneration is unknown. The aim of the present study was to investigate whether BMP-7 can prevent TNF-α- or H_2O_2-induced apoptotic effects in cultured human disc cells obtained from degenerated discs.

Materials and methods

Cell culture

NP tissues were freshly collected from eight subjects undergoing lumbar total disc replacement surgery (age: 48±16 years). Informed consent was obtained from subjects under approval of the South-Eastern Health Service Human Research Committee, Sydney, Australia. All discs demonstrated moderate signs of degeneration on magnetic resonance imaging including decreased water content and decreased disc height. Discarded NP tissues were immediately subjected to 0.025% collagenase digestion overnight. Primary cultures were grown in a complete medium containing Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA), 10% fetal calf serum, and 1% penicillin/streptomycin for 10 to 12 days to become confluent. Cells were subcultured at a concentration of 1×10^5/mL for 2 to 3 days before treatment. All experiments were completed using the second passages of cells.

Apoptosis induction and BMP-7 treatment

Apoptosis was induced with either TNF-α (B&D System, Inc., Minneapolis, MN) or H_2O_2 (Sigma-Aldrich, St. Louis, MO). TNF-α apoptotic induction was performed with 100 ng/mL for 24 hours and 48 hours. H_2O_2 apoptotic induction was conducted with 0.1 mM for 20 minutes. Human recombinant pure BMP-7 (rhBMP-7; Stryker-Biotech, Andover, MA) at a concentration of 100 ng/mL was preadministrated in cultures for 5 hours and remained in the medium during TNF-α or H_2O_2 treatment. A dose-response study was performed before determining the rhBMP-7 dose for this study (data not shown). The dose of BMP-7 used in the present study was further selected based on a number of published studies on cells from IVDs [23,24]. Cells grown in complete medium alone served as the control.

In situ detection of DNA fragmentation

In situ detection of DNA fragmentation in apoptotic cells was performed with the terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick end labeling (TUNEL) kit (Promega, Madison, WI) as per the manufacturer's instructions. Briefly, the cells cultured on coverslips were fixed in 4% paraformaldehyde and treated with proteinase K for 15 minutes. The endogenous peroxidase was blocked with 3% H_2O_2 for 10 minutes. The cells were incubated with TdT for 2 hours and then exposed to antidigoxigenin antibody conjugated with peroxidase. Color was developed with 3,3′-diaminobenzidine hydrochloride. Slides were then counterstained with hematoxylin. Negative controls were incubated with reaction mixture lacking TdT enzyme. Cells were defined as apoptotic when the whole nuclear area was labeled brown. The counts were performed in three different sets of experiments for each condition with 500 cells.

Cell viability assay

Cell survival was measured with the MTS Cell Proliferation Assay kit (Promega, Madison, WI) using 1×10^4 cells/well plated in 96-well plates. Assays were performed as specified by the manufacturer, such that only viable cells are able to metabolically reduce tetrazolium salts to formazan salts, detected directly on a spectrophotometer at 490 nm.

Caspase-3 activity

Caspase-3 activity was detected using the CaspACE assay colorimetric system (Promega, Madison, WI) according