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Event details:

International Society for the Study of the Lumbar Spine (ISSLS 2006)
Norway

Publication Date:

2006

DOI:

<https://doi.org/10.26190/unsworks/752>

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ISSLS - 2006

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Bone Morphogenetic Protein rescues human Intervertebral Disc cells *in-vitro* from apoptosis

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Introduction: 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. The aim of this study was to determine whether BMP-7 rescues cultured intervertebral disc cells following stimulation of apoptosis.

Methods: Nucleus pulposus tissue were obtained from consent individuals under surgical procedures and digested with collagenase prior to culturing. Apoptosis induction was achieved with either TNF- α or H₂O₂ incubation. BMP-7 (Stryker) was used at 100 ng/ml, 5 hours prior to the addition of apoptotic stimulators. Cellular apoptosis was detected by TUNEL assay, caspase-3 activity and caspase-3 protein expression. Cellular proliferation and viability was assayed by H³-thymidine incorporation. Collagen II and aggrecan protein levels were measured using western blots and immunostaining. Alkaline phosphatase activity and nitric oxide was measured.

Results: Both extrinsic and intrinsic apoptotic pathways were induced by TNF- α and 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 a complete block of the apoptotic effects of both inducers as well as the nitric oxide induced by TNF- α . BMP-7 increases cellular viability, proliferation and extracellular matrix production in an apoptotic environment with no osteoblastic activity induction of discal cells

Discussion

BMP-7 retards apoptosis of cultured human disc cells induced by either tumor necrosis factor-alpha or hydrogen peroxide. Induction of apoptosis lead 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.