

Oxidative stress induces expression of markers of early osteoarthritis and chondrocyte terminal differentiation in cultured bovine explants

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INTRODUCTION: Articular cartilage is susceptible to many forms of injury, some of which may lead to secondary arthritis at a much later time. Free radicals are formed by articular cartilage in response to injury, by chondrocytes themselves or through inflammatory pathways. We have examined the behavior of chondrocytes embedded within their native matrix to oxidant stress following exposure to hydrogen peroxide (H₂O₂). In addition to observing degradative effects of oxidant stress we have attempted to examine the synthetic reactions that occur as a result of repair and remodelling.

METHODS: Bovine articular cartilage explants (obtained from the metatarsalphalangeal joint of 18 month-old steers) were cultured in DMEM, 10 mM HEPES, insulin-transferin-selenium and gentamycin. Explants were treated with a single dose of 0.1, 0.5 and 1.0 mM H₂O₂, the culture medium was changed after 24 hours and subsequently after every third day. Immunohistochemical and immunofluorescence labelling of control and treated explants was performed using mouse anti-3B3(-), rabbit anti-procollagen type IIA, mouse anti-PCNA and mouse anti-nitrotyrosine antibodies.

RESULTS: Bovine explants were treated with a single application of 0.1, 0.5 and 1.0 mM for 7 to 21 days. Presence of the oxidant was undetectable in the culture medium within 6 hours of its addition. Cell death occurred in a statistically significant dose-responsive manner with increasing concentrations of H₂O₂ in serum-free medium. Addition of ITS to the culture medium also led to a dose-dependent increase in cell death but significantly less than was observed in serum-free cultures. There was evidence of cellular proliferation in explant cultures. PCNA positive nuclei were detected in treated explants and treated explanted were also positive for gene expression of cyclin b2 part of the mitosis-promoting factor in dividing cells. In contrast control explants were negative for PCNA labelling and cyclin b2 expression.

3B3(-) is an antibody that recognises an atypical change in the biochemical structure of chondroitin sulphate chains covalently attached to aggrecan. Reactivity for 3B3(-) occurs in the normal fetal growth plate and articular cartilage undergoing osteoarthritic changes. We found that treatment of explants with H₂O₂ induced expression of the 3B3(-) epitope in the superficial and upper middle zones, Figure 1. Moreover we discovered that expression of the 3B3(-) epitope progressively increased over a period of 3 weeks (with no further addition of H₂O₂), at which time labeling was intense and throughout the territorial matrix in the surface and middle zones of the explants.

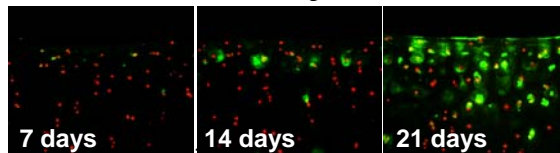


Figure 1. Progressive increase in 3B3(-) labelling over time following 0.5mM H₂O₂ treatment.

Procollagen IIA that is expressed during limb development and re-expressed in OA was observed in treated explants. Procollagen IIA antibody labelling correlated with increased collagen type II gene expression. Our work has also shown that late stage OA in age matched animals is accompanied by reduced expression of 3B3(-) epitopes and a reduction in procollagen IIA labelling, indicating that failure of repair processes either through cell loss or dedifferentiation may presage irreversible decline in joint function..

DISCUSSION & CONCLUSIONS:

Oxidatively-stressed cartilage explants recapitulate many of the earliest molecular signatures of early OA, whether this represents a transient repair process or a sustained degenerative process remains to be seen. Oxidant injury therefore represents a useful, reproducible *in vitro* experimental model to study molecules critical in repair and progression of cartilage degeneration.

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