GADODIAMIDE INCREASES PROTEOGLYCAN PRODUCTION AND INDUCES APOPTOSIS IN CULTURED CHONDROCYTES

*Greisberg, J; *Wolf, J; *Wyman, J; *Zou, L; +*Terek, R
+*Brown University Department of Orthopaedics, Providence, RI. UOI, MOC Suite 200, 2 Dudley Street, Providence, RI 02905, 401-457-1555, Fax: 401-831-8992, Richard_Terek@Brown.edu

Introduction: Magnetic resonance arthrography, a procedure which utilizes intra-articular gadolinium containing contrast agents, has become a useful tool in musculoskeletal diagnosis. Although gadolinium is considered safe for systemic use, toxicities in some tissues and cells have been identified. In this study, toxicity of gadolinium on cartilage was assessed by measuring the effect of gadolinium on proteoglycan synthesis, cell proliferation, and apoptosis in cultured chondrocytes. The hypothesis is that gadolinium is not an inert substance with respect to cartilage metabolism.

Methods: Chondrocyte Isolation: Articular bovine cartilage was harvested under sterile conditions, minced, and digested with collagenase. Cells were plated at 5x10^5 per cm^2 and after 7 days in culture exposed to gadodiamide at varying concentrations for 16 hours. The 1X dose was 4mmol/L, the concentration frequently used for arthrography. Controls were cultured without gadodiamide.

Proteoglycan Production: Cultured cells were incubated with [35S] sulfate to label newly synthesized proteoglycan. Labeled proteoglycan was separated from lysed cultures using chromatography. Radioactivity in the exclusion volume, representing newly synthesized proteoglycan, was measured in a liquid scintillation counter.

Thymidine Incorporation: Prior to exposure to gadolinium, the cultures were synchronized by incubating the cells in serum-free medium for 24 hours. Cultured cells were then incubated with serum containing media and [3H] thymidine to label proliferating cells. DNA was precipitated from lysed cells with 7.5% trichloroacetic acid in 0.2% bovine serum albumin. The DNA pellet was washed with 10% trichloroacetic acid and then dissolved in 0.1M sodium hydroxide. Radioactivity, representing newly synthesized thymidine, was measured in a liquid scintillation counter.

Apoptosis Assay: Cultured cells were analyzed for apoptosis with the TUNEL assay, in which nucleotide labeled with fluorescein is incorporated into DNA strand breaks of apoptotic cells. The percent of apoptotic cells was measured under fluorescent microscopy.

Data Analysis: The results of both the proteoglycan production and proliferation assays were normalized to the number of cells in each culture by measuring total DNA from the cell lysates using a fluorometric dye assay. Statistical significance was tested for each assay by Kruskall-Wallis 1-way analysis of variance and the student’s t-test. A p value of < 0.05 was considered significant.

Results: Incorporation of labeled sulfate (Figure 1) in the 1X and 5X test groups was higher than in the control group (p=0.026 and 0.048, respectively), while the 10X group was similar to control (p=0.95).

Thymidine uptake in the 10X test group was lower than control (Figure 2, p=0.009).

Conclusions: Gadodiamide increases proteoglycan production, decreases proliferation, and induces apoptosis in cultured chondrocytes. Gadodiamide is therefore not inert in these in vitro assays. The reason for the increased proteoglycan production is not clear, but may represent a stress response. The net effect on the matrix would depend on the combined effects of proteoglycan synthesis and degradation. The effects on cell proliferation and apoptosis are concerning. If gadolinium is toxic to chondrocytes, long-term deleterious effects on matrix integrity could result. Further work is needed to determine the impact of gadolinium contrast agents on cartilage matrix and chondrocyte viability in the intact joint.

References: