Effects of Chronic Rotenone Treatment on Cartilage Responses to Impact Injury

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Introduction. The acute effects of blunt impact injury to articular cartilage include chondrocyte death and extracellular matrix damage, which contribute to the pathogenesis of post-traumatic osteoarthritis (PTOA). Previous studies in our laboratory showed that impact injury induces cell death by eliciting superoxide release from mitochondria, a process that continues for at least 24 hours after injury. Oxidant release in the first few hours after impact and cell death at 48 hours post-impact were blocked by acute treatment (0-24 hours post-impact) with rotenone, an inhibitor of the mitochondrial electron transport chain. However, rotenone should also reduce energy production from mitochondrial respiration, which may promote cartilage degeneration by undercutting biosynthetic activity in surviving cells. To test this, we studied the effects of chronic exposure to rotenone (daily for 7 days) on impacted and normal cartilage in bovine osteochondral explants. Effects on viability, protein biosynthesis, and proteoglycan content were evaluated on the 7th day of culture. To distinguish between cell-sparing effects and metabolic inhibition, treatment in one group was delayed until 48 hours post-impact, a time when most cell death had already occurred.

Materials and Methods. Osteochondral explants (2.5 x 2.5 cm) were harvested from bovine lateral tibial plateaus and incubated in culture medium under standard conditions. Some explants received a single blunt impact (7 J/cm²) to the cartilage surface using a custom built drop tower device with a 5-mm diameter platen.

Rotenone treatment: Explants were treated with rotenone (2.5 µM) daily starting immediately after impact or starting 48 hours after impact (delayed treatment). Viability: Calcein AM and ethidium homodimer stained cells were imaged in situ using a confocal microscope. Two fields inside and outside impact sites were imaged at a 20X magnification. Tissue Harvest: Cartilage samples were harvested from each explant using a 4-mm dermal punch after 7 days in culture. Metabolic Activity: Protein synthesis was measured by measuring 1H-Proline incorporation for 18 hours of radiolabeling. The results were normalized to the wet weight of the samples (CPM/mg tissue). Proteoglycan Content: Proteoglycan (PG) content was measured by standard Dimethylmethylen Blue (DMMB) assay methods. Values were normalized to the wet weight of the samples (µg GAG/mg tissue). Statistics: Statistical analysis was performed using a Two Way ANOVA with a post Holm-Sidak correction for multiple comparisons (p < 0.05).

Results.

![Figure 1. Effects of Rotenone and Impact on Chondrocyte Viability.](image1)

Figure 1. Effects of Rotenone and Impact on Chondrocyte Viability. Columns and error bars represent means and standard deviations based on 5-6 explants. Control viability (No Impact, No treatment) was >95%. Rotenone itself (No Impact, Tx) had no significant effect (p = 0.322). Impact without rotenone (Impact, No Tx) significantly reduced viability compared to control (p < 0.001). Viability in the impacted group treated with rotenone immediately after impact (Impact, Tx) was significantly higher than in impact only group (p < 0.001), but viability in the delayed rotenone treatment group (Impact, Tx Delayed) was significantly lower than in the impact only group (p < 0.001).

![Figure 2. Effects of Rotenone and Impact on 1H-Proline Incorporation.](image2)

Figure 2. Effects of Rotenone and Impact on 1H-Proline Incorporation. Columns and error bars show mean counts per minute per mg of tissue (CPM/mg tissue) and standard deviations based on 5-6 explants. Rotenone alone significantly inhibited incorporation (p = 0.004). Incorporation was reduced by impact, but the difference was not significant (p = 0.103). Incorporation in impacted explants treated immediately with rotenone was similar to control and significantly higher than in the impact, no treatment group (p = 0.001) and the impact delayed treatment group (p < 0.001).

![Figure 3. Effects of Rotenone and Impact on Proteoglycan Content.](image3)

Figure 3. Effects of Rotenone and Impact on Proteoglycan Content. Columns and error bars represent means and standard deviations based on 5-6 explants. Relative to control, rotenone had no effect on PG content (p = 0.065), but PG content in impacted explants was significantly lower than control (p = 0.044). Explants that were impacted and treated immediately with rotenone showed significantly higher PG content than impacted explants that were not treated (p < 0.001), but PG content in impacted explants with delayed rotenone treatment was not different than in untreated explants (p = 0.267).

Discussion. In non-impacted cartilage chronic exposure to rotenone had no effect on viability or proteoglycan content, but did significantly impair proline incorporation, suggesting that mitochondrial respiration played a role in supporting matrix biosynthesis. Chronic rotenone treatment begun immediately post-impact blocked impact effects on all three outcome measures, but delaying treatment for 48 hours did not change impact effects, indicating that the benefits of rotenone treatment were due to inhibition of acute cell death in the first 24 hours following impact. This resulted in an overall increase in metabolic activity due to higher cell densities at impact sites despite inhibition of respiration. Taken together these results suggest that injured cartilage stability may be significantly improved by blocking acute cell death even if mitochondrial function in surviving cells is impaired.

References
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2) Goodwin et al. Trans ORS, 2009: 671

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