Effects of Cytoskeletal Inhibitors on Reactive Oxygen Species and Viability in Chondrocytes Following Blunt Impact Injury to Osteochondral Explants

INTRODUCTION:
Blunt impact to interarticular cartilage and other joint injuries can lead to the pathogenesis of post-traumatic osteoarthritis (PTOA). Excessive impact-induced release of reactive oxygen species (ROS) has been shown to lead to chondrocyte damage and death. Previous studies in our lab have shown that the initial release of ROS following blunt impact to cartilage is produced by the mitochondria. It has been shown that mechanical deformation of a cell leads to displacement of mitochondria due to cytoskeletal changes, but cells treated with cytoskeletal inhibitors do not experience appreciable displacement of their mitochondria. We hypothesized that cytoskeletal inhibition may shield mitochondria from impact-induced stress, thereby limiting ROS production and saving chondrocytes. To address this, we studied the effect of cytochalasin B, a microfilament inhibitor, and nocodazole, a microtubule inhibitor, on impact-induced ROS production and viability.

METHODS:
Osteochondral Explants: Osteochondral explants (2.5cm x 2.5cm x 1.0cm) were harvested from bovine lateral tibial plates. Explants were maintained overnight at 37°C in culture medium (10% Fetal Bovine Serum (FBS), Dulbecco Modified Eagle Medium (DMEM), Ham F-12) under low oxygen conditions (5% O2, 5% CO2). The next day, explants were transferred to low O2 equilibrated Phenol Red-Free culture medium (10% FBS, 1:1 DMEM/F12) and incubated overnight under the same low O2 conditions.

Treatment and Impact: The following day, explants were treated with either 20μM cytochalasin B (Calbiochem) (n=8) or 10μM nocodazole (Sigma) (n=8) for four hours under low O2 conditions. No treatment control explants (n=7) were incubated in low O2 conditions until impact. Explants were subjected to a 7 J/cm² impact injury post treatment. Oxidant Production and Viability Imaging: Directly after impact, explants were fluorescently probed with dihydroethidium (DHE), a superoxide probe, and Calcein AM, a live cell probe, to study the effects of treatment on ROS production. During imaging, explants were submerged in low O2 equilibrated DMEM/F12. Explants were imaged using a 1024 Bio-Rad Confocal Microscope. After imaging, explants were incubated overnight in fresh Phenol Red-Free medium under low O2 conditions. Twenty-four hours after impact, explants were stained with ethidium homodimer-2, a dead cell stain, and Calcein AM to study viability. Three sites within the impact site and three sites ~0.5cm outside the impact site were imaged during each imaging session.

Data Analysis: Kruskal-Wallis One Way ANOVA with Dunn’s pairwise comparison was used to assess the effects of the different treatments on DHE staining and viability.

RESULTS:
Confocal images taken inside and outside the impact sites were analyzed using ImageJ to determine the percent of DHE positive cells after impact and viability 24 hours after impact (Figure 1).

Figure 1. ROS production and viability in impact sites of cytochalasin B and nocodazole explants and control sites of no treatment explants. The top row of micrographs shows DHE-stained cells (red nuclei) and Calcein AM-stained cells (green cytoplasm) in the impact sites and a control site of an untreated explant. Viability micrographs of Calcein AM-stained cells and ethidium homodimer-2 stained cells (red nuclei) are shown in the second row. Scale bar denotes 50 microns.

Impact sites of the cytochalasin B treatment group had a significantly lower percentage of DHE stained cells than the no treatment impact group (p<0.05) (Figure 2). There was not a significant difference between the impact sites of the nocodazole treated and no treatment explants; however, DHE staining in the nocodazole treated explants (24.9%) was lower than in the no treatment explants (42.4%). The percentage of ROS producing cells in control sites of the no treatment group (21.7%) was significantly lower than in the group’s impact sites (42.4%). Also, there was no significant difference between any of the control site groups.

Figure 2. ROS production by chondrocytes in impacted and control osteochondral explants after treatment and impact. Significantly lower ROS production was measured in the cytochalasin B impact site than in the no treatment impact site. The only treatment group that showed a significant difference between viability in its control (80.1%) and impact (60.8%) sites (p<0.05) was the no treatment group (Figure 3). There was a significant difference in viability between the impact sites of the cytochalasin B (80.0%) and the no treatment groups and the nocodazole (79.9%) and no treatment groups (p<0.05). No significant difference between impact site viability of the cytochalasin B and nocodazole treated groups was observed. Also, there was no significant difference between any of the control groups.

Figure 3. Viability of chondrocytes after treatment and 24 hours after impact. Significantly higher viability was measured in the impact sites of cytochalasin B and nocodazole treated explants than the impact sites of no treatment explants.

DISCUSSION:
Inhibition of chondrocytes’ cytoskeleton by cytochalasin B and nocodazole reduce the production of ROS after a blunt impact injury. Although not significant, nocodazole treated impact sites show a decrease in oxidative stress. With the reduction of ROS, chondrocytes within impact sites of explants treated with cytoskeleton inhibitors experience superior viability when compared to those in untreated impact sites. Also, cytoskeleton inhibition did not adversely affect chondrocyte viability. These findings support the hypothesis that mechanical stress stimulates release of ROS from mitochondria and cytoskeleton inhibition exhibits a cell sparing effect.

REFERENCES:

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