PRESERVATION OF CELL VIABILITY IN CARTILAGE EXPLANTS FOLLOWING SEVERE BLUNT TRAUMA

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Introduction:

Severe blunt trauma to a joint has been associated with the development of chronic diseases such as osteoarthritis (OA).[1] While the various mechanisms of post-traumatic OA are largely unknown, traumatic insults on chondral and osteochondral explants can result in matrix damage and cellular death.[2] Cell death alone can be responsible for tissue changes indicative of early OA.[3] Apoptotic cell death has also been associated more frequently with OA cartilage than normal tissue in the clinical setting.[4] Following a blunt trauma to cartilage, the percentage of dead cells has been shown to increase significantly in the first 24 hours.[2] Recently, early death of chondrocytes under cyclic mechanical loading was shown to be due to necrosis.[5] A leaky cell membrane and the inability to maintain cellular homeostasis are characteristics of necrosis. Blunt impact loading on joints produces shear stresses that may damage cell membranes and cause necrotic cell death.[6] Nonionic synthetic surfactants have been shown to seal cell membranes and rescue cells from necrotic death in skeletal muscle and brain tissue.[7,8,9] The hypothesis of the current study was that early administration of Poloxamer 188, an 8.4 k-Da nonionic synthetic surfactant, would help preserve the viability of chondroctyes in the first 24 hours post blunt trauma. These data may suggest a potential early intervention for a traumatized joint that would help limit the extent of cell death and mitigate a post trauma disease process.

Methods:

Bovine forelegs were obtained from a local abattoir within six hours of slaughter and skinned prior to opening the metacarpal joint under a laminar flow hood. A biopsy punch was used to make 6 mm diameter chondral explants. The specimens were equilibrated for 48 hours in DMEM:F12 supplemented with 10% fetal bovine serum in a 37° incubator (5% CO_2, 95% humidity). Sixteen explants were loaded to 850 N (~ 30 MPa) at a low rate of loading (1 s to peak) in unconfined compression. The loading of the explants was performed between two highly polished stainless steel plates using a servo-hydraulic testing machine (Instron, model 1331, Canton, Ma). Eight explants were frozen in optimal cutting temperature media at four time points out to 96 hours, sliced using a cryotome into 0.8 mm slices and adhered to glass slides. The slices were stained for apoptosis by established methods (Deadend Fluorometric TUNEL System, Promega, Madison, WI). The remaining eight explants were sliced into 0.5 mm sections and stained with Calcein AM and Ethidium Homodimer to determine cell viability (Live/Dead Cytotoxicity Kit, Molecular Probes). Thirty-two explants were loaded to 707 N (~25 MPa) using a low rate of loading in unconfined compression. Eight mg/ml Poloxamer 188 was added to the media of 16 explants. The remaining explants were assigned to a no Poloxamer group. Eight explants from each group were randomly selected and used to determine cell viability at either 1 or 24 hours post impact. The explants were stained for cell viability according to the above procedure. The stained explants from both experiments were viewed in a fluorescent microscope and photographs were taken with a digital camera. Each explant slice was then divided into three zones: superficial (top 20%), middle (middle 50%), and deep (bottom 30%). A blinded observer using image software (Sigma SCAN, SPSS INC., Chicago, IL) quantified either apoptosis or cell viability in each explant zone. The percentage of dead cells in each zone was calculated and a two-factor ANOVA was used as a statistical test between groups. Statistical significance was indicated at $p<0.05$.

Results:

In the first experiment the blunt impact at 30 MPa did not cause apoptotic cell death in chondrocytes; rather cellular necrosis was prevalent in all zones of the explant. In the second experiment cell death in the superficial zone was concentrated around surface fissures (Figure 1A). In treated specimens many of these cells were rescued (Figure 1B). The percentage of cell death in untreated explants increased significantly between 1 and 24 hours (Figure 2 A, B, C). In contrast there was not a significant change in the percentage of cell death in any zone for the Poloxamer 188 treated samples. In fact, there was a significant decrease in the percentage of cell death in Poloxamer 188 treated explants at 24 hours versus untreated explants within all zones of the explant (Figure 2 A, B, C). Interestingly, there was also a tendency ($p>0.05$) for explants treated with the surfactant to have fewer dead cells at one hour post trauma than the untreated explants.

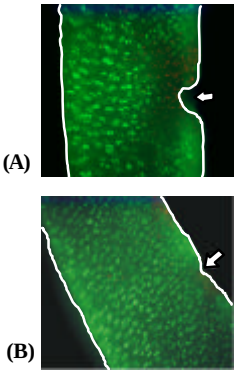

Figure 1: Untreated explant at 24 hours post-trauma (**A**) and Poloxamer 188 treated explant at 24 hours post-trauma (**B**). Note a significant increase in viable cells around fissures (arrows) in the treated group.

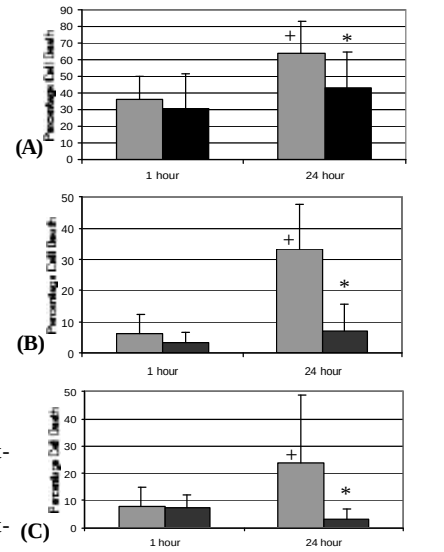

Figure 2: Percentage cell death at the superficial (**A**), middle (**B**) and deep (**C**) zones. Specimens Untreated (■) and Treated with Poloxamer 188 surfactant (■). (*) signifies a decrease versus untreated explants. (+) signifies an increase over the 1 hour group.

Discussion:

This study documented a significant increase in cell death between 1 and 24 hours post trauma, via a necrotic pathway. The experiment more importantly documented the ability of Poloxamer 188, a nonionic surfactant, to significantly preserve the viability of chondrocytes post trauma. Importantly, early administration of the surfactant helped preserve cell viability around fissures as well as throughout the tissue explant. This surfactant has been shown to effectively patch damaged areas of the cell's plasma membrane and help preserve cell viability until normal cellular repair can occur.[10] The surfactant is then 'squeezed out' of the membrane and excreted unchanged in the urine following cellular repair. Since Poloxamer 188 has been shown not to affect apoptotic cell death, the experiments further support the work of Chen et al, suggesting early cell death via a necrotic pathway. This study therefore suggests that Poloxamer 188 may be useful as an early intervention to rescue cells from acute traumatic death. The long term consequences of rescuing these cells *in vivo* will need to be evaluated in future studies using our established animal model of post traumatic OA.

Acknowledgment:

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References:

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