CELLULAR SIGNALING AS A CAUSE OF CELL DEATH IN CYCLICALLY IMPACTED CARTILAGE EXPLANTS

*Levin, A; +*Burton-Wurster, N; **Chen, C; *Lust, G
+*James A. Baker Institute, Cornell University, Ithaca, New York. Hungerford Hill Rd., Ithaca, NY 14853, (607) 256-5651, Fax: (607) 256-5608, niw1@cornell.edu

Introduction

Mechanical damage to the cartilage matrix is a known cause of osteoarthritis (OA). In vitro cartilage studies have shown that impact loading can produce structural damage and OA-like changes, including tissue swelling and collagen denaturation (2,4,6). Last year, we reported that repetitive impact loading also induced cell death in the damaged cartilage after 2 days of post-impact culturing (5). It is our hypothesis that injured chondrocytes at the site of impact release a diffusible signal which moves through the cartilage, resulting in the spread of cell death over time from impacted to non-impacted regions of our samples. By extending the periods of post-impact culturing to 3, 6, and 21 days, we were able to show increases in cell death over time as well as a locational spreading of dead cells from impacted to non-impacted regions of cartilage. Furthermore, by physically separating the non-impacted regions from the impacted regions of the same cartilage sample for culture, we were able to prevent cell death in the non-impacted regions, lending support to our hypothesis.

Materials and Methods

Cartilage explants were obtained from the articular surface of canine shoulders as 4mm disks of uniform thickness. Animal use and necropsy followed the procedures approved by our Institutional Review Board (protocol no. 84-94-96). Cyclic impacts were applied to the 2mm core of the 4mm disk, and 16 samples were applied at 0.3Hz, with one second of active loading per cycle. After impacting, cartilage disks were cultured separately from the impacted cores, the degree of cell death was not statistically different from the core region's 68.8% cell death in the non-impacted ring regions reached 66.5%.

Whole-disk samples cultured for 21 days after 120 minutes of impacting, the impact loading also induced cell death in the damaged cartilage after 2 days of post-impact culturing (5). It is our hypothesis that injured chondrocytes at the site of impact release a diffusible signal which moves through the cartilage, resulting in the spread of cell death over time from impacted to non-impacted regions of our samples. By extending the periods of post-impact culturing to 3, 6, and 21 days, we were able to show increases in cell death over time as well as a locational spreading of dead cells from impacted to non-impacted regions of cartilage. Furthermore, by physically separating the non-impacted regions from the impacted regions of the same cartilage sample for culture, we were able to prevent cell death in the non-impacted regions, lending support to our hypothesis.

Results

Our initial findings verified previous reports of increased cell death with increased duration of impacting (5), and also showed an increase in cell death with prolonged duration of post-impact culturing. The data presented in Fig. 1 indicate the significant increases in dead cell percentages at 6 days and 21 days over those of 3 days of post-impact incubation. Within the first three days after impacting, the majority of TUNEL+ cells were in the impacted core (23.9±4.2% in the core after 120 minutes, 12.7±2.4% in the surrounding ring). However, after extended culture for 21 days, cell death did not remain localized to the regions of the cartilage disk that were directly impacted. Rather, in whole-disk samples cultured for 21 days after 120 minutes of impacting, the percent cell death in the non-impacted ring regions reached 66.5±8.4%, which was not statistically different from the core region's 68.8±6.7%.

When the ring regions were separated from the cores immediately after loading and cultured separately from the impacted cores, the degree of cell death in these unloaded areas was as low as in non-loaded controls, while the separated cores continued to exhibit high levels of cell death (Fig. 2).

Discussion

Our initial observations that cell death increased with the duration of post-impact culturing, and that the location of the dead cells spread from the damaged regions to the surrounding unloaded regions over time, suggest that a diffusible cell death signal may be released at the site of damage and permeate to the peripheral areas. Our ability to prevent cell death from occurring in these surrounding regions by physical isolation from damaged cartilage, lends credence to the hypothesis that diffusible signals are involved. Previous studies have suggested that much of the cell death observed within the first two days in cyclically impacted cartilage could be due to necrosis, in addition to the apoptotic episodes (5). The nature of the secondary wave of cell death, as well as the signals responsible, are currently under investigation.

A reduction in chondrocyte numbers is one characteristic of OA. A striking loss of chondrocytes in the early phases of cartilage degeneration has been a reproducible histochecmical observation of long standing in the hip dysplasia model of OA (9), and cell numbers were reduced in early lesion cartilage despite the appearance of multicellular clusters (3). Vignon, et al (10) has reported that reduced chondrocyte density preceded focal cartilage fibrillation in a surgically induced model of OA in the rabbit. More recently, increased apoptosis has been associated with human OA (1,8). Thus, a loss of chondrocyte viability may play a significant role in the progression of OA, and the possible finding that intercellular signaling is involved in the early onset of chondrocyte loss may play a significant role in the progression of OA, and of controlling the expression of osteoarthritis.

References


Dr. Steven Bloom’s contributions to this work are gratefully acknowledged.