Anti-apoptotic treatments prevent cartilage degradation after acute trauma to human ankle cartilage.

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INTRODUCTION

There is a clear need to recognize the risk of osteoarthritis (OA) following joint injury, and to develop and implement strategies to prevent post-traumatic OA. Our overall hypothesis is that the OA which is subsequent to acute trauma is caused by: 1) initial death and apoptosis of chondrocytes adjacent to, and within, the injury site and 2) the failure of these cells to mount a robust reparative response. The aim of this part of study was to investigate if anti-apoptotic agents (a membrane stabilizing non-ionic surfactant P188 and inhibitors of caspase 3 and 9) applied immediately following acute injury will reduce the development of post-traumatic cartilage degeneration and promote cell survival after a single impact to human ankle cartilage.

METHODS

Ten morphologically normal tali obtained from human organ donors (50-70 years old) within 24 hours of death through the Gift of Hope Organ & Tissue Donor Network were impacted with 1 Ns generating peak forces in the range of 600 N using a 4mm diameter indenter. Non-impacted control cartilage was obtained from the subtalar joint. 8mm diameter cartilage plugs consisted of 4mm diameter impacted core and 4mm adjacent ring and the non-impacted control were removed and cultured as explants with or without P188 (8mg/ml), caspase 3 (10 uM) or 9 (2umM) inhibitors for 48 hrs. Results were assessed in the superficial and middle-deep layers together and separately at days 0, 2, 7, and 14 after injury by live-dead cell assay, apoptosis (Tunel stain), histology with Safranin O-fast green staining and Mankin score and proteoglycan (PG) content in the media by DMMB assay. Statistical analysis was done with non-parametric ANOVA to compare all subgroups (p<0.05 was considered statistically significant). Unpaired t-test was used to compare each group with the non treated control group (p<0.05).

RESULTS

After impaction, the number of live cells was immediately decreased especially in the superficial layer of the impacted cores. In culture, if untreated, cells continued to die and by day 14 cell survival was reduced by more than 30% in the superficial layer in comparison with day 0 (p<0.05). No significant changes were observed in the middle/deep cartilage zone. Among treatments, P188 appeared to be more effective in preventing cell death by necrosis than caspase inhibitors. In comparison with the non-treated control, cell death by apoptosis was significantly reduced by P188 already at day 2 in the superficial layer of both core (36% vs 11%) and ring (13% vs 8%) respectively. This effect was maintained during 14-days culture and constituted a 2-fold difference in the number of positive cells in the untreated control; p<0.05). Caspase-9 inhibitor did not improve histological appearance of cartilage sections.

The impact also induced cell death by apoptosis, which was observed in the impacted core and ring in all cartilage layers. With culture, apoptosis spread out to the areas that did not experience the impact and by day 14 there was a statistical increase in the level of apoptosis in the core and ring of the impacted control. P188 showed the strongest effect on apoptosis among all three treatments. In comparison with the non-treated control, cell death by apoptosis was significantly reduced by P188 already at day 2 in the superficial layer of both core (36% vs 11%) and ring (13% vs 8%) respectively. This effect was maintained during 14-days culture and constituted a 2-fold difference in the number of tunel-positive cells between non-treated and P188-treated cores (Fig. 3A) and rings (Fig.3B; P<0.05). Both anti-caspase-3 and 9 were effective in the reduction of apoptosis, but only in the superficial layer of the core area and only in the first 7 days.

DISCUSSION

The results of the present study demonstrate that a single impact to human articular cartilage ex vivo resulted in cell death by necrosis and apoptosis, cartilage degeneration, and radial progression of cell death to the areas adjacent to the impact. Early intervention with P188 specifically and caspase 3 inhibitor to a lesser extent were effective in promoting chondrocyte survival and thus protecting cartilage from degenerative changes. P188 was the only treatment to decrease cell death in both core and ring areas and to prevent the expansion of cell death to the non-impacted areas. Combination of these anti-catabolic treatments with anabolic agents may provide novel therapeutic approaches to stimulate proper cartilage repair after acute trauma and in order to prevent post-traumatic OA.

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Figure 1. Representative images of live/dead cell assay in the non-treated and P188 treated impacted core and ring areas taken at day 2 (A-B, E-F) and day 14 (C-D, G-H) of culture. A & C, non-treated impacted core; B & D, non-treated impacted ring; E & G, P188 treated core; F & H, P188 treated ring.

Figure 2. Photomicrographs of cartilage explants stained with Safranin O. A & B, non-treated impacted control; C & D, P188 treated specimens. A & C, Day 2 culture; B & D, Day 14 culture.

Figure 3. Graphical representation of Tunel-positive cells in the core (A) and ring (B) (*p<0.05 in comparison with the corresponding non-treated control; †p<0.05 between treatments by ANOVA).