Inhibitors of Mitogen Activated Protein Kinases Rescue Chondrocytes from Blunt Impact-Induced Cell Death in Cartilage

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INTRODUCTION: Post-Traumatic Osteoarthritis (PTOA) is initiated by joint injury and is characterized by progressive articular cartilage deterioration. Loss of chondrocytes, the sole cell type in cartilage, has been observed in injured cartilage and chondrocyte death contributes to cartilage degeneration. Thus, preventing losses in cellularity could help to reduce the risk of PTOA. In last year’s meeting we reported that p38 mitogen activated protein (MAP) kinase was activated 20 minutes after a single blunt impact on cartilage. The activation then radiated outward to adjacent cartilage within 24 hours. Another MAP kinase, ERK1/2, was also activated in both impacted and surrounding cartilage within 24 hours. Since both p38 and ERK1/2 have been implicated in mediating apoptosis, we hypothesized that specific inhibitors of these two MAP kinases would ameliorate chondrocyte death in cartilage injured by a single high energy blunt impact. To test this we determined the effects of p38 and ERK1/2 inhibitors on chondrocyte viability in an explant injury model.

METHODS: Pre-impact culturing: Osteochondral plugs (25 mm X 25 mm X 10 mm) were obtained from mature bovine stifle joints and equilibrated in DMEM/F12 containing 10% FBS overnight at 37 °C, 5% CO2, and 5% O2. The plugs were then pre-incubated with 10 μM SB202190, 10 μM SB203580, or 10 μM U0126 for 90 minutes. Blunt impacting: The plugs were impacted with a drop tower constructed to deliver impact energy density of 14 J/cm2 to a 5.0 mm brass rod indenter resting on the cartilage surface. Fresh medium with inhibitors were re-applied to the impacted plugs every other day. Confocal microscopy: At day 7, osteochondal plugs were stained with ethidium homodimer and calcine AM. A BioRad 1024 Confocal Microscope was used for taking three Z-section images from the impact zone and three from the areas immediately to the impact zone (annulus). Each experiment was repeated at least three times. The ratio of live to total cells was averaged and plotted. The significance was assessed by using Dunnett multiple comparisons test.

RESULTS: The p38 MAP kinase inhibitor, SB20190, greatly increased live cell percentage in both impact zone and surrounding cartilage. The number of live cells (green) was dramatically increased in both the impacted and annulus cartilage while the number of dead cells (red) was greatly reduced in cartilage treated with SB20190 when compared to the corresponding areas in cartilage without SB20190 (Figure 1). The average viability of impact and annulus sites from the impacted only explants was 61.5% and 60.9%, respectively. While with the treatment of SB20190, the viability was increased to 87.7% in the impact sites and 83.8% in the annulus sites, which were close to the percentages in the non-impacted controls or cartilage treated with the inhibitor only. The difference was statistically significant (Figure 2).

DISCUSSION: The specific inhibitor to p38 and ERK1/2 MAP kinases effectively blocked blunt injury induced cell death not only on the impact zone but in the surrounding cartilage. The live cell ratio was increased by these two inhibitors to the levels similar to that in the non-impacted control cartilage. Combining with our previous observation of the activation of these two MAP kinases in cartilage following a single blunt impact, these results might suggest the involvement of p38 and ERK1/2 MAP kinases in blunt impact induced cell death pathways in chondrocytes. Furthermore, these findings suggest that early inhibition of these two MAP kinases might help to prevent cartilage from deterioration in mechanically injured cartilage.

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