INTRODUCTION

Cyclic load is an important factor for cartilage metabolism. A number of studies have shown that cyclic load increases the biosynthesis of proteoglycans, collagen, and other proteins in articular cartilage [5,7,10], but little information exists about the effects of load on injured cartilage or cartilage defect. A classic study by Salter et al. [8] suggested that continuous passive motion ameliorated the healing of trabecular cartilage defects, but immobilization of the joint produced adverse surgical outcomes. Several recent in vitro studies suggest that cyclic load can affect cytokine induced changes through NF-kB and other pathways and is closely associated with cartilage catabolism [2, 3, 6]. However, the effects of mechanical load on cartilage degradation after injury are not clear. Several studies of joint injury suggest that cartilage degeneration is closely related to the upregulation and activation of matrix metalloproteinases (MMPs) including MMP-3 and MMP-13 [4]. MMP-13 is one of the collagenases to cleave type II collagen in articular cartilage and has been shown upregulated in early stages of osteoarthritis, while MMP-3 can activate MMP-13 and cleave aggrecan core protein. The objective of this study was to determine whether intermittent daily load increases collagen cleavage after load injury. Our hypothesis was intermittent cyclic load at 1.0MPa would upregulate MMP-13 and would increase collagen cleavage in the injured cartilage.

MATERIAL AND METHODS

Cartilage explants were harvested from the trochlear groove of immature (<2 month) bovine knees using a 7-mm diameter biopsy punch, trimmed to a 1-mm uniform thickness, and cultured with DMEM supplemented with ITS+ supplement for at least two hours. Culture medium was collected at day 1, 4, 7 after initial injury. On day 7, explants were harvested and sectioned to determine for cell viability as previously described [1]. Explants in the loaded and Injury+Load group received cyclic compression at 1.0MPa for 3hrs/day for seven days. Culture medium was measured after rehydrated in culture medium for at least two hours. Hypotonic wet weight was measured after rehydrated in distilled water for two hours. Dry weight was measured after lyophilized overnight. Location of collagen cleavage and MMP-13 were immunohistochemically stained determined using COL2-3/4Cmax (kindly provided by Dr. Robin A. Poole) and MMP-13 (Chemicon) antibodies as previously described [1]. Proteoglycan content in the culture medium was determined using the DMMB assay. The effects of the treatment were evaluated by one-way ANOVA or Student’s t-test using Systat (10.2, SPSS). P-value less than 0.05 was considered significant.

RESULTS

There was no increase of cell death in the Load group as compared to the controls while cell death in the superficial and middle zones was consistent found in the center (directly injured) area in the Injury and Injury+Load groups. A significant increase of water content was found in the Injury+Load group as compared to Injury alone (Fig. 2B vs. 2D). The staining in the Injury+Load explant is particularly localized in the superficial zone where cell death occurred. The localized increase of collagen cleavage was also correlated well with the upregulation of MMP-13 in the Injury and Injury+Load groups, which suggested that the upregulation of MMP-13 was, in part, responsible for the breakdown of collagen network (Fig. 3). No changes in PG content in culture medium were found between groups. No significant changes of biomechanical properties between treatments were found while a marginal decrease of aggregate modulus and a marginal increase of hydraulic permeability were found in the Injury+Load explants (p=0.08 and 0.06) as compared to the controls (data not shown.)

DISCUSSION

The increase of hypotonic swelling in degraded cartilage is commonly thought due to the further swelling of proteoglycan in the disrupted collagen network. The collagen degradation is induced in part by the accumulation and activation of collagenase, as suggested by collagen cleavage and MMP-13 staining. Intermittent cyclic load at 1.0MPa (normal joint load ranging from 1 to 10 MPa) for 3 hours each day can induce further collagen breakdown and cartilage degradation. These biochemical and immunohistochemical findings support our hypothesis that intermittent daily load increases collagen degradation in injured cartilage. There, however, exist no changes in the PG content and biomechanical properties, which may be due in part to the fact that only a small portion of explant (~18%) is received direct injury. This finding suggested that post-injury load at the physiological level modulates the upregulation and activation of collagenase and that mechanotransduction is important for post-injury cartilage degradation as well as the onset of secondary osteoarthritis.

REFERENCES