INTRODUCTION

Traumatic joint injury is a known risk factor for development of osteoarthritis, but the mechanism is not well understood. This has led to the development of in vitro models to investigate the immediate effects of injurious compression of the cartilage tissue (1). Previous studies have revealed several effects of injurious compression on chondrocytes, including apoptotic cell death and loss of anabolic response to dynamic compression (2-4). While investigators have documented an increase in GAG loss from cartilage matrix after injury, this loss has generally not been either a large proportion of total GAG content, or sustained over time. In addition, although the focus of an injury model is on catabolic activity, we hypothesize that the failure of anabolic repair and/or protection responses is also important to understand. We have therefore studied two aspects of the response of normal adult human cartilage to injurious compression: 1) the interaction between injury and exogenous interleukin-1α (IL-1) addition and 2) the effect of injury on the levels of endogenous osteogenic protein-1 (OP-1, BMP-7) protein.

METHODS

Normal human donor articular cartilage was obtained within 48 hours of death through collaboration with the Regional Organ Bank of Illinois from donor joints of patients with no history of joint disease. Articular joint cartilage was taken from sites that included the knee femoropatellar groove, femoral condyles, and tibial plateau, as well as the ankle talotibial and subtalar joints. Only cartilage which appeared smooth and uncalcified was used. Cartilage was drilled out, sliced, and punched to produce explant disks 3 mm in diameter and 0.5 to 1.0 mm in thickness, depending on the total depth of the cartilage. All experimental groups were matched for cartilage location and depth by using as evinced in 1 M guanidine and nitrogen. The conditioned medium was saved and assayed for sulfated GAG content by DMMB dye. Cartilage tissue was extracted in 1 M guanidine and treated with proteinase K for 24 hours. The amount of protein in the supernatant was determined by the method of Lowry et al. (5). The conditioned medium was saved and assayed for sulfated GAG content by DMMB dye. The amount of protein in the supernatant was determined by the method of Lowry et al. (5).

RESULTS

Figure 1. Knee Cartilage

Results are first shown for an experiment in which both knee and ankle joints were obtained from the same donor, a 72-year-old man with Collins grade 2 injured knee cartilage (2.8 µg), and little observed effect of 10 ng/ml IL-1α alone (Fig. 1). In contrast, cartilage that was injured and then cultured in IL-1β was found to release more than twice the average amount of GAG as controls (an increase of 7.1 µg). The interaction between injury and IL-1α was statistically significant in a linear regression model (p=0.01). In ankle cartilage, the loss of GAG from uninjured control disks was 4.2 ± 0.3 µg. Compared to controls, injury had no observed effect on GAG loss from ankle cartilage (Fig. 2). There was a small increase in mean GAG loss after incubation with IL-1α, similar to the effect seen in knee cartilage. The two treatments combined, injured cartilage incubated in IL-1α, resulted in a small increase in mean GAG loss, but the interaction between IL-1α and injury was not statistically significant (p=0.50).

To further clarify the effect of injury in human ankle cartilage, 18 explant disks from 4 donor ankles were injured at different levels of strain and analyzed for GAG loss. The mean GAG loss from injured cartilage was less than that of controls by 0.4 ± 0.3 µg after injury (not significant), and there was no significant association between GAG loss and either peak stress (R²=0.02, p=0.6) or strain rate (R²=0.12, p=0.16). Regarding our second study, an age-matched control from five donor knee joints was analyzed for OP-1 content after injury (Fig. 3). In cartilage from all five donor knees, the average OP-1 protein content was increased three days after injury by at least 50% (p = 0.06 for comparison to free-swelling controls).

DISCUSSION

Investigation of the effects of injurious compression on human articular cartilage found that the cytokine IL-1α can act synergistically with injury to produce loss of sulfated GAG from the cartilage matrix. This result expands upon our previous finding of a similar synergistic action of injury with IL-1α in newborn bovine femoropatellar groove cartilage (5). It is possible that this synergistic between results from sensitization of the chondrocytes by injury to cytokine stimulation. It is also possible that injury causes mechanical disruption of the matrix such that at the molecular level, proteolytic enzymes released by cytokine action have more favorable access to their target substrates. In either case, the extension of this finding to human knee cartilage suggests this effect as a possible mechanism for interaction between mechanically injured cartilage and enzymatic matrix degradation. It is also interesting to note that unlike human and bovine calf knee cartilage, the human ankle cartilage showed no increase in GAG loss after relatively high levels of compression, and only a suggestion of a synergistic response to IL-1α and injury. This is consistent with the hypothesis that the lower incidence of ankle OA compared to knee OA may in part be due to differences in cell response to cytokines, mechanical forces, or growth factors (7). A previous report has shown that the talotibial ankle cartilage has a higher GAG content and mechanical stiffness in comparison to knee cartilage (8), which could contribute to increased tolerance for compressive loads. Thus, the data presented here for IL-1α activity in response to injury motivate an additional hypothesis in our investigations to identify differential catabolic and anabolic responses to injurious compression. We also show preliminary evidence for increased levels of mature OP-1 protein after injury in human knee cartilage. Previous studies have identified endogenous OP-1 in adult human normal and osteoarthritic cartilage and documented the chondroprotective and anabolic activity of exogenous OP-1 in several conditions (6). It has also been shown that there is an age-related decrease in OP-1 content (9), and our ongoing studies will seek to confirm the effect of both injury and donor age on upregulation of OP-1 content.

REFERENCES


ACKNOWLEDGMENTS

The Regional Organ Bank of Illinois and the donors’ families are gratefully acknowledged. The authors also thank Arcady Margulis, MD for assistance with human donor cartilage. This work was funded in part by NIH grants AR45779, AR39239, and AR47654, and a grant from Stryker Biotech (KK-001).

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49th Annual Meeting of the Orthopaedic Research Society

Poster #0695