INCREASED RELEASE OF PROTEOGLYCAN AND STROMELOYSIN-1 (MMP-3) AND DECREASED RELEASE OF NITRIC OXIDE IN LOAD-INJURED CARTILAGE

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INTRODUCTION

The initial events of cartilage matrix degradation are important for the onset of osteoarthritis (OA) and may be closely related to the local elevation of matrix metalloproteinases (MMPs) and nitric oxide (NO) [1,2,3]. Our previous studies have shown an increase of cell death and collagenase-cleaved collagen in the superficial zone of cartilage explants after excessive cyclical loads [4,5]. Our changes in proteoglycan (PG) degradation and loss after mechanical loads are not clear. The objective of this study was to determine the release of PG, NO, and MMP-3 from the cartilage explant after load-induced injury and the accumulation of MMP-3 in situ with post-load incubation.

METHODS

Cartilage explants were excised from the loaded region of mature bovine humeral heads (1.5-2 years) within 4 hr post-mortem and incubated in serum-free DMEM until used as previously described [5]. Explants (n=5 in each group) were loaded in confined compression at 1 and 5 MPa for 0.17, 1, 6 or 24 hours [5]. Additional explants were used as non-loaded controls. After load removal, the explants were cultured for an additional 24 hours. The culture media was collected, and the explants were digested in papain solution at 65°C overnight. The culture media were analyzed for PG, nitric oxide, and MMP-3 content using the 1,9-dimethylmethylen blue dye-binding method, Greiss assay, and MMP-3 assay (Chemicon, Temecula, CA), respectively. The digested explant was also analyzed for PG content. Additional explants were loaded and cultured for 24 and 48 hours and examined for immunohistochemical staining of MMP-3. Human OA tissue that was obtained from a patient with total-knee replacement, following the IRB protocols, was used as a positive control for MMP-3. Six μm slides were cut and blocked in 10% normal goat serum before they were incubated at room temperature for 1 hour with the primary antibodies against MMP-3 (Chemicon). After washing, slides were incubated with a secondary goat-anti-mouse antibody conjugated to FITC (fluorescein) and kept in the dark for 1 hr at room temperature. Gelvatol, an anti-fade reagent, with PI for counter-staining cell nuclei was used to mount the cover slips. The effects of loading on the PG, NO, MMP-3 were determined by two-way ANOVA using commercially available software. Data are presented as mean ± SEM. The statistical significance level (a) was set at 0.05.

RESULTS

Our results showed increased PG release into the media from explants loaded with 1 MPa for 0.17, 1 and 6 hour as compared to the nonloaded control (Fig. 1A). No change in PG release was found in explants loaded with 1 MPa for 24 hour. Also, no changes were found in the groups loaded with 5MPa versus non-loaded controls (Fig. 1A). A significant decrease in PG release was measured in the group loaded with 5MP for 24 hour. The NO content in the culture media increased significantly after load removal (p<0.001, ANOVA; Fig. 1B). This correlated with a decrease in NO content in the explants loaded with a high magnitude of dynamic load [3]. There was an increase of MMP-3 content in the media of explants loaded with 5 MPa (p<0.001, ANOVA, Fig. 1C). However, no change of MMP-3 content was found in the group loaded with 1 MPa. Using the immunohistochemical assay, we found the MMP-3 staining in the explant loaded with 5MPa for 1 hour increased with the time of post-load incubation (Figs. 2B-2C). There was no staining in the non-loaded control and a strong staining in human OA cartilage (Figs. 2A,2D).

DISCUSSION

Our results indicate that a moderate load/injury increases the PG release to the media, and that a severe injury reduces the PG release to the media. The decrease of NO release might be closely related to the matrix degradation and cartilage repair as previously suggested by Fermor et al. [3]. Our quantitative analysis showed an increased release of MMP-3 from explant due to load-induced injury. Immunohistochemical staining of MMP-3 was localized in the topmost layers. Our finding of increased MMP-3 content is consistent with the previous finding that an increase of MMP-3 in the synovial fluid of OA patients as well as in OA cartilage [1].

This suggests that the load-injured cartilage undergoes matrix degradation similar to that of OA cartilage. Although the role of MMP-3 is not completely clear, MMP-3 has been shown to activate MMP-1 and MMP-13 in vitro. A recent study by Lark et al. showed the presence of MMP-3 with MMP-degraded aggrecan (by using an antibody against VDIPEN neoepitope) in the cartilage from patients with OA and RA [6]. Of more interest, the MMP-3 is concentrated in the superficial region where collagen cleavage and cell death was found in our previous study [5]. This suggests that MMP-3 might play an important role in the proteolytic degradation of the cartilage.

REFERENCES


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