Synergistic effect of hyaluronan and intermittent hydrostatic pressure on MMP-13 mRNA expression in osteoblasts from osteoarthritic subchondral bone

Introduction

Periarticular osseous changes such as subchondral bone sclerosis and osteophyte formation occur during the progression of OA. It is reported that various inflammatory cytokines and proteases are involved in the initiation and progression of OA. Matrix metalloproteinase-13 (MMP-13) is known to be implicated in bone resorption, as well as a main catabolic enzyme for the collagen type II in the cartilage matrix (1). We previously reported that the expression of MMP-13 enhances in osteoarthritic subchondral bone (2).

Intraarticular injection of hyaluronan (hyaluronic acid, HA) is a symptom-modifying approach for reducing pain due to OA and widely utilized clinically. The disease modifying mechanisms of HA in osteoarthritic cartilage and synovium are widely reported, however, the effect of HA in osteoarthritic subchondral bone remains unclear. The purpose of this study is to investigate the effect of HA and mechanical stress on the gene expressions of protease in osteoblasts isolated from osteoarthritic subchondral bone.

Methods

OA subchondral bone from the distal end of the femur was harvested from 6 patients (mean age; 81.2 ± 7.2) at total knee arthroplasty. The subchondral bone underlying the articular cartilage was cut into small pieces and incubated in DMEM for 3 weeks, and then osteoblasts were isolated.

Subchondral bone osteoblasts (SBOs) were cultured with DMEM containing 30% fluorescent labeled HA for 48 hours and washed twice with PBS. The monolayer cultured cells were observed with a fluorescence microscope. As control, SBOs cultured without fluorescent labeled HA was used.

SBOs obtained from 6 patients were divided into 4 experimental groups. Control group: cultured without stimulation, HA group: incubated with HA (1000 mg/ml, 48 hours), IHP group: applied intermittent hydrostatic pressure (IHP) (1/2Hz, 5MPa, 60minutes), and HA+IHP group: incubated with HA followed by IHP. Total RNA were extracted and mRNA expression was examined by real-time RT-PCR for MMP-13.

This study was approved by the local ethics committee of Kyoto Prefectural University of Medicine. Written informed consent was obtained from all patients.

Statistical significances between control group and the other groups were analyzed by one-sample t-test.

Results

In the fluorescent labeled HA group, fluorescence was observed in the area of cytoplasm but not in nucleus 48 hours after the administration (Figure 1).

The mRNA expressions of MMP-13 in the HA group was 103.1 ± 27.2% compared to those in the control group. In the IHP group, MMP-13 mRNA expression was reduced to 76.6 ± 26.3%. In the HA+IHP group, MMP-13 mRNA expression was suppressed to 54.8 ± 12.7% and there was a significant difference compared to the control group (p<0.016) (Figure 2).

Conclusions

The role of subchondral bone attracts attention in the onset and/or progression of OA. It was reported that HA effectively decreases subchondral bone density and thickness and changes trabecular structure toward rod-like, and that subchondral bone becomes more compliant and thereby reduces cartilage stress (3). However, the mechanism of the influence of HA on subchondral bone remains unclear.

In this study, HA was exposed to osteoblasts, and the enhanced expression of MMP-13 in osteoarthritic osteoblasts was significantly suppressed by HA in combination with IHP. In the natural course of OA, a resorption of subchondral bone was documented in the early stage of OA and was considered to take an important role in the progression of the disease. In OA, neovascularization between cartilage and subchondral bone is observed while tidemark disappears. Therefore, intra-articular injected HA could reach the subchondral tissue. The results in this study suggest that intra-articular injection of HA in combination with appropriate exercise could suppress MMP-13 expression in subchondral bone, which may prevent abnormal metabolism in osseous tissue in OA.