Hyaluronan and intermittent hydrostatic pressure synergistically suppressed MMP-13 and IL-6 in osteoblasts from osteoarthritic subchondral bone

Introduction

Various inflammatory cytokines and proteases are involved in the initiation and progression of osteoarthritis (OA)\(^1\). We previously reported that the expressions of matrix metalloproteinase-13 (MMP-13) and interleukin-6 (IL-6) were augmented in OA subchondral bone\(^2\). The objective of the present study was to investigate the effects of hyaluronan (hyaluronic acid, HA) and mechanical stress on osteoblasts isolated from OA subchondral bone.

Methods

OA subchondral bone from the distal end of the femur was harvested from 9 patients at total knee arthroplasty. The subchondral bone underlying the degenerated 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 were divided into 4 experimental groups. Control group : cultured without stimulation, HA group : incubated with HA (1000 mg/ml, 48 hours), IHP group : intermittent hydrostatic pressure (IHP) (1/2Hz, 5MPa, 60minutes), and HA+IHP group : incubated with HA followed by IHP. Total RNA was extracted and mRNA expression was examined by real-time RT-PCR for MMP-13 and IL-6. In control group and HA+IHP group, culture supernatant was harvested 24 hours after the application of HA + IHP, and concentrations of IL-6 and MMP-13 were measured using an enzyme-linked immunosorbent assay (ELISA).

Values were analyzed statistically by Tukey-kramer’s test and paired t-test, and a value less than 0.05 was considered significant.

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

RESULTS

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

The mRNA expressions of MMP-13 of the HA group, the IHP group and the HA+IHP group compared to the control group were 101 ± 18.2 %, 89.3 ± 14.1 % and 51.2 ± 7.5 %, respectively, indicating that MMP-13 expression in the HA + IHP group significantly decreased compared to those in the control group and in the HA group. The IL-6 mRNA of those groups were 76.6 ± 11.9 %, 73.2 ± 10.5 % and 54.0 ± 18.3 %, indicating that IHP treatment and HA + IHP treatment significantly suppressed the IL-6 mRNA(Figure 2).

The production of MMP-13 and IL-6 were 51.3 ± 11.6 (pg/ml) and 70.8 ± 25.6 in the control group. In the HA+IHP group, they were significantly reduced to 53.9 ± 25.6 and 45.2 ± 11.1 (pg/ml) (Figure 3).

DISCUSSION:

The role of subchondral bone attracts attention in the onset and/or progression of OA. It was reported that HA influences metabolism in subchondral bone, 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 expressions of MMP-13 and IL-6 in OA osteoblasts were 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 and IL-6 expressions in subchondral bone, which may prevent abnormal metabolism in osseous tissue in OA.

REFERENCES: