NOTE  Surgery

Preventive Effect of Hyaluronic Acid on the Suppression of Attachment and Migration Abilities of Bovine Chondrocytes by IL-1α in vitro

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ABSTRACT. Attachment and migration of bovine chondrocytes cultured in vitro were significantly suppressed by the addition of interleukin (IL)-1α at the concentration of 1 ng/ml or more (p<0.05). The application of hyaluronic acid (HA) at the concentration of 10 µg/ml or more significantly recovered the attachment of chondrocytes (p<0.05) and the application of HA at 100 µg/ml concentration recovered the migration of chondrocytes suppressed by IL-1α. These results suggest that the application of HA for inflammatory arthropathies or chondrocyte transplantation might be helpful to preserve the properties of chondrocytes and its extracellular matrix against inflammatory conditions.

KEY WORDS: bovine chondrocyte, hyaluronic acid, interleukin-1α.


Articular cartilage in adult animals is an avascular, aural and alymphatic tissue [19, 23]. Damaged articular cartilage has usually a very limited potential for repair, and degraded cartilage metabolites induce synovitis and further degeneration of the articular surface. Over the last decade, autologous chondrocyte implantation has been reported to be an ideal method to accelerate the regeneration of damaged cartilage and reestablish the articular surface with hyaline cartilage [6, 7, 9]. Chondrocytes have abilities to attach to and migrate into extracellular matrix (ECM) constructed with fibronectin, chondronectin, and type I and II collagens [14, 22]. A number of studies have suggested that migration, attachment and proliferation of chondrocytes or chondrogenic cells in the damaged area play an important role to enhance the regeneration of cartilage [20, 25]. These abilities of chondrocytes are however suppressed by nitric oxide (NO) which is induced by inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor [8].

Hyaluronic acid (HA), a linear polysaccharide and nonsulfated glycosaminoglycans (GAG), is found in the extracellular and the pericellular matrix of many tissues and synovial fluid [13]. In the joint, HA is also the nucleus of the proteoglycan aggregates in the ECM of articular cartilage and lubricates the articular surface as a synovial component. In vitro studies revealed that HA enhances chondrocyte proliferation and GAG synthesis and inhibits cartilage degradation [11, 17].

The purpose of this study was to evaluate the effects of HA on chondrocytes attachment to and migration into ECM under IL-1α supplementation in bovine articular chondrocytes in vitro.

As experimental animals, four clinically healthy cattle were used in this study immediately after death at the local slaughterhouse. Full thickness of articular cartilage was aseptically harvested from the surface of carpal and tarsal joints. The cartilage was washed with sterile physiological saline, containing 50 µg/ml gentamicin, and then cut into small pieces. Cartilage pieces were digested with 1 mg/ml collagenase (Wako Pure Chemical Co., Osaka, Japan) in Dulbecco’s modified Eagle medium (DMEM; Gibco BRL, Grand island, NY, U.S.A.), containing 63.5 µg/ml penicillin and 100 µg/ml streptomycin, for 18 hr at 37°C under gentle stirring. Isolated chondrocytes were filtered through a 50 µm nylon mesh and centrifuged at 2000 x g for 5 min. The pellet was resuspended and cultured with DMEM, containing 10% fetal bovine serum, using 100-mm plastic tissue culture dishes (Corning Co., Corning, NY, U.S.A.) for 2 weeks. Chondrocyte cultures were performed at 37°C in a 95% air and 5% CO2 humidified atmosphere. The culture medium was changed twice a week. At confluence, chondrocytes were harvested after 0.25% trypsin (Nacalai Tesque Inc., Kyoto, Japan) treatment, and prepared at 1 x 106 cells/ml in DMEM for the following examinations. Cell viability was estimated by a trypan blue exclusion test.

The attachment ability of cultured chondrocytes was assayed by using a tissue culture plate coated with collagen type II. Two hundred µl of collagen type II solution (30 µg/ml, porcine cartilage, Wako Pure Chemical Co.) in phosphate buffered saline (PBS, pH 7.4) was added to each well of a 96-well tissue culture plate (Corning Co.), and stored overnight at 4°C. Wells were then washed once with PBS, and incubated with 1 ml bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) in PBS for 2 hr at room temperature. The plate was used for the following examinations after washing one time with PBS. Hyaluronic acid (Molecular weight: 9.5 x 106), prepared from rooster combs, was kindly given from Seikagaku Kogyo Co. (Tokyo, Japan) and recombinant human IL-1α was purchased from Genzyme Inc. (Cambridge, MA, U.S.A.). Chondrocytes (1 x 105 cells) were cultured for 1 hr with IL-1α (0, 0.01, 0.1, 1 or 10 ng/ml), or mixture of HA (1, 10 or 100 µg/ml) and IL-1α (1 ng/ml) in DMEM in wells prepared
as described above. Three application procedures of HA and IL-1α were employed as follows; A) HA was added into wells and incubated for a few min, then IL-1α was supplemented, B) HA and IL-1α were added at the same time, and C) IL-1α was added and incubated for a few min, then HA was supplemented. After unattached chondrocytes were removed by washing with PBS, chondrocytes were fixed with absolute methanol, and stained with 0.2% crystal violet solution for 30 min. After washing 3 times with a distilled water, they were solubilized with 1% sodium lauryl sulfate (Wako Pure Chemical Co.) in distilled water. An absorbance (570 nm) was measured with a plate reader (MTP-120, Corona Electric, Ibaraki, Japan) in triplicate.

The migration ability of cultured chondrocytes was assayed by using transwell chambers (8 µm-diameter pore size filters in 6.5-mm, Corning Co.). Chondrocytes were cultured with IL-1α (0, 1 or 10 ng/ml), or mixture of HA and IL-1α in DMEM. The lower chambers were filled with different concentration of HA (0, 1, 10 or 100 µg/ml) diluted in DMEM, containing 30 µg/ml type II collagen and 1 ng/ml IL-1α. The upper chambers were filled with chondrocytes in DMEM (1 × 10^5 cells/100 µl of DMEM), containing same concentrations of HA and IL-1α as in each lower chamber. The cells were incubated for 6 hr at 37°C. After removing cells from the upper chambers and on the upper surface of the membrane, cells on the lower surface of the membrane were fixed with absolute methanol and stained with 0.2% crystal violet or with hematoxylin and eosin. The number of cells on each membrane was counted in nine randomly chosen microscope fields at the magnification of 400-fold, and the mean and standard deviation were calculated.

The data obtained from control and other groups were compared using Mann-Whitney U test at a statistically significant level of p<0.05.

The viability of isolated chondrocytes was always more than 95%. The chondrocyte attachment ability was significantly decreased by the supplementation of IL-1α at the concentration of 1 ng/ml or more, compared with the control (p<0.05, Fig. 1).

Chondrocytes migrated through the membrane are shown in Fig. 2. The chondrocyte migration was suppressed by IL-1α at the concentration of 1 ng/ml or more, compared with the control, and decreased in a dose-dependent manner according to the concentration of IL-1α (Fig. 2).

The addition of HA at the concentration of 10 µg/ml or more significantly recovered the attachment ability of chondrocytes suppressed by IL-1α (p<0.05, Fig. 3). The addition of HA before IL-1α supplementation seems to be more effective in the chondrocyte attachment than that after IL-1α supplementation (Fig. 3).

The addition of HA at the concentration of 100 µg/ml significantly recovered the migration ability of chondrocytes suppressed by IL-1α, compared with the control (p<0.05, Table 1).

The present study showed that IL-1α suppressed the attachment and migration abilities of chondrocytes in vitro. Inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor are considered to play major roles in the inflammatory joint disease. These cytokines stimulate the release of metalloproteinases and prostaglandin E2 from chondrocytes.
and synoviocytes, and promote the degradation of ECM, which is the important matrix for cell adhesion in the cartilage [2, 4, 12, 15, 18]. Some of these effects have been associated with oxygen radicals and NO induced by inflammatory cytokines. It was reported that these inflammatory products might accelerate necrosis and apoptosis of chondrocytes [5], and that NO may disrupt the formation of actin stress fibers of chondrocytes. This indicates that these products would suppress the attachment and migration of chondrocytes [8].

The concentration of 1 ng/ml of IL-1α was used for the stimulation of chondrocytes in the examination of both chondrocytes attachment and migration activities. It was reported that 1 ng/ml IL-1α was enough to inhibit the synthesis of proteoglycans from chondrocytes in vitro [1, 16]. In this study, a significant IL-1α-induced suppression of chondrocytes attachment was confirmed at the concentration of 1 ng/ml or more (Fig. 1).

The chondroprotective effects of HA were reported to scavenge oxygen-derived free radicals [21], to inhibit chondrocytes apoptosis [24] and to suppress chemotaxis and phagocytosis of macrophages and granulocytes in arthritic joint [3]. It is therefore expected that HA could preserve properties and activities of chondrocytes against IL-1α.

In this study, the dilutions of HA were adjusted to concentrations under 100 µg/ml. These concentrations were usually employed for the analysis of HA bioactivities [11]. Higher concentration of HA would be physically inhibitory in attachment and migration abilities of chondrocytes due to its high viscosity.

It was demonstrated here that HA recovered the chondrocyte attachment ability, which was suppressed by IL-1α, and that the application of HA before IL-1α supplementation seems to be more effective than the application of HA after IL-1α supplementation. These chondroprotective effects on the attachment to collagens indicated that HA reduced IL-1α effects on chondrocytes as described above. The fact may also suggest that a physical property of viscosity of HA is an additional factor against IL-1α on the chondrocyte attachment.

The application of HA significantly recovered or improved the chondrocyte migration ability, which was suppressed by IL-1α in this study. It was demonstrated that HA created hydrous channels, which could facilitate cell migration in the extracellular matrix [26]. More recently, it was reported that the network structure in fibrin gels became alveoli with large pores by the addition of HA, and this gels strongly stimulated cell migration [10]. These findings may support a hypothesis that HA has supportive effects on cell migration in its extracellular matrix. On the contrary, the short-time migration assay employed here, would be suitable for the evaluation of the direct effect of HA on each cultured chondrocyte. It is therefore suggested that HA has a chondroprotective effect against IL-1α on migration.

In conclusion, the present study suggests that the HA application for inflammatory arthropathies, or as a supplement agent for implantation of chondrocytes or chondrogenic cells against damaged cartilage, is of value to preserve the properties of chondrocytes and its extracellular matrix against inflammatory state in the joint.

REFERENCES