Effects of hyaluronic acid and basic fibroblast growth factor on motility of chondrocytes and synovial cells in culture

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ABSTRACT – The capacity of chondrogenic precursor cells to migrate and proliferate in an injured area is considered to be essential for cartilage repair. We examined cell motility of chondrocytes and synovial cells in monolayer culture and the chemokinetic effects of hyaluronic acid (HA) and basic fibroblast growth factors (bFGF) on these cells.

The velocity of chondrocyte migration was accelerated by giving bFGF and simultaneously administering of both HA and bFGF, but it was not affected by HA alone. The velocity of synovial cell migration was increased by HA, but not by bFGF. HA had a chemokinetic effect on synovial cells and bFGF had the same effect on chondrocytes. Treatment with exogenous HA and bFGF may be of value for repairing articular cartilage injury by recruiting chondrogenic cells and promoting migration of chondrocytes in the cartilage tissue.

■

The capacity of articular cartilage repair itself is limited (Campbell 1969, Furukawa et al. 1980) and partial thickness defects in the articular cartilage do not heal spontaneously (Meachim 1963, Mankin 1974). Two hypotheses have therefore been proposed. There are no blood vessels in the mature articular cartilage (Calandruccio and Gilmer 1962, Mankin 1974) and the articular cartilage does not have access to stem cells in bone marrow, which induce cartilage repair (Wakitani et al. 1994). Recent studies have suggested that the capacity of chondrogenic precursor cells to migrate and proliferate in an injured area may be important for cartilage growth and repair (Reindel et al. 1995, Hunziker and Rosenberg 1996) and some cytokines and growth factors are known to stimulate chondrocytes to proliferate or to produce cartilage matrix. While most studies of the relationship between growth factors and the articular cartilage have focused on the metabolic effects on chondrocytes, less is known about the effect on the reparative processes, such as recruitment of precursor cells from synovium and cell migration into the cartilage tissue.

In this study, we directly observed cell migration during administration of a growth factor using phase contrast microscopy and a videodisc recorder as well as a chemotactic chamber to examine the effects of bFGF and HA on the chemokinesis of chondrocytes and synovial cells.

Animals and methods

Isolation of chondrocytes

The number of animals was kept to a minimum, and the experiments were made according to the stipulations of the Ethics Committee for Experimental Animals of Shimane Medical University. Articular cartilage slices were taken from the proximal humerus, distal femur, and proximal tibia of 4 10-week-old Japanese white rabbits. They were cleared of adherent connective tissue and cut into small pieces. Chondrocytes were then isolated by enzymatic digestion (Wolff et al. 1992). Briefly, cartilage specimens were minced and washed 3

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times in sterile saline before isolating the chondrocytes first with 0.25% trypsin in sterile saline for 30 min followed by 0.25% collagenase in DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin (100 IU/mL)/streptomycin (100 μg/mL)/amphotericin B (0.25 μg/mL) for 4–8 hrs at 37 °C in a culture bottle.

Isolation of synovial cells

Synovial membrane was taken from the knee joints. It was washed 3 times with sterile saline, cut into small pieces, and then transferred into DMEM. Synovial cells were obtained by explant culture from the synovial membrane.

Measurements of spontaneous migration velocity

Chondrocyte or synovial cell suspensions from 3 animals were then plated in DMEM buffered to pH 7.3 and supplemented with 10% FBS and antibiotics (5×10^2 cells/cm^2). After the cells had become attached and spread (for approximately 2 hrs), the plate (Falcon 3001, Becton Dickinson, NJ, USA) was placed on the stage of an OLYMPUS IX 70 phase-contrast inverted microscope for time-lapse measurements using a SONY LVR-3000 AN time-lapse videotape recorder for 4 hrs. The time-lapse observations were made with the cells in the presence of bFGF (human, recombinant, Progen, Heidelberg, Germany) (0.01, 0.1, 1, 10 ng/mL), HA (MW about 80K Da, Seikagakukogyo, Tokyo, Japan) (0.01, 0.1, 1 mg/mL) or the simultaneous administration of both bFGF and HA. A calibration scale for distance was obtained by taping a graded scale placed in a cultured dish. The velocity of migration of the cells was determined by measuring the position of each cell, represented by the center of the nucleus, at different times on the monitor according to Ferrier et al. (1994). To examine the inhibition effect on chondrocyte migration, bFGF solution (10 ng/mL) was preincubated for 30 min at 37 °C with anti-bFGF antibody (anti-human bFGF, gout-polyclonal, R&D System, USA) (50 μg/mL). We did 6 independent experiments to calculate significant differences.

Chemokinesis assay

Chemokinesis assays were done using a chemotaxis chamber, Chemotaxicell (polycarbonate filters with pore sizes of 8 μm, Kurabo, Tokyo, Japan). Basic FGF was prepared in DMEM at a concentration of 0.01, 0.1, 1, 10 ng/mL and added to a tissue culture plate (Multiwell, 24 wells, Becton Dickinson, NJ, USA). HA was prepared at a concentration of 0.01, 0.1, or 1 mg/mL. Basic FGF and HA were give separately is various concentrations for chemotaxis assay as well. Each chemotaxis chamber was put on a multiwell culture plate. A 200-mL chondrocyte suspension and synovial cell suspension, with a cell density of 5×10^5 cells/mL in DMEM, was added to the chemotaxis chambers. The chambers were placed in an incubator at 37 °C for 1 hr. After incubation, the filters were dismantled, and the upper surface of the filter was wiped with a cotton swab. Chondrocytes and synovial cells on the lower surface of the filter were fixed in 70% ethanol for 5 min at room temperature. The cells were stained with toluidine blue and viewed under a microscope. The number of cells that migrated to the lower surface of the filter, per mm^2, was counted 6 times on each filter using a 1-mm^2 eyepiece grid, and the average was determined. The chemotactic activity of cells was normalized by the control well in which only culture medium was added to the lower chamber, in accord with a previous report (Chan et al. 1997), where chemotactic activity (%) = number of cells per mm^2 in presence of growth factors/number of cells per mm^2 in control well × 100 (%). Data were expressed as mean chemotactic activity (%) with standard deviation. We made 6 independent experiments to assess differences.

Statistics

Results were expressed as the mean plus or minus the standard deviation (SD). One-way analysis of variance was used to analyze the results. The Student Newman-Keul's test was used for multiple comparisons among the control and treatment groups. Differences of $p < 0.05$ were considered significant.

Results

Chondrocytes and synovial cells were cultured in low population to maintain open spaces and the cells just before and after mitosis were removed.

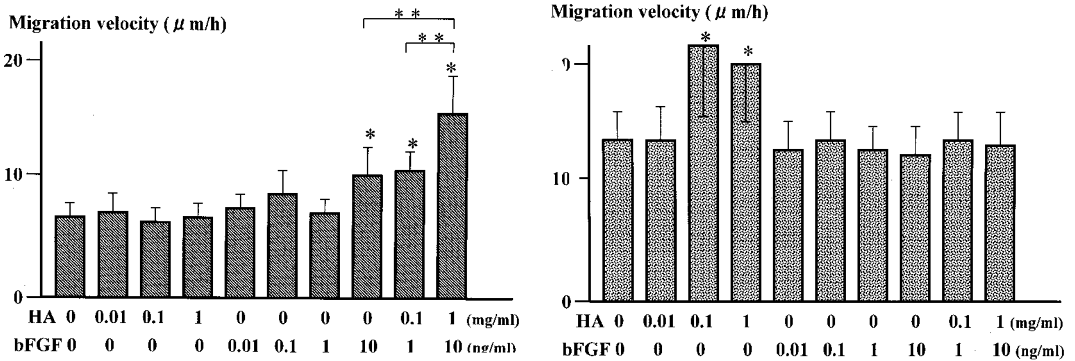

Figure 1. Effects of HA and bFGF on chondrocyte migration (left) and synovial cell migration (right). Results are expressed as the mean velocity of migration with standard deviation. Statistically significant differences between the control and experimental groups are indicated by * ($p < 0.05$), ** ($p < 0.01$).

Chondrocytes tended to be polygonal in appearance in these conditions and developed many short projections from the surface of the cell and migrated at random with the projections moving actively. In the presence of HA and bFGF, the appearance of chondrocytes did not change during observations.

Most of the synovial cells showed a bipolar or trigonal morphology and migrated with their processes on the cell extending eccentrically. In the presence of HA and bFGF, synovial cells did not change their appearance during our observations.

The spontaneous migration velocity of synovial cells exceeded that of the chondrocytes (13.6, SD 4.3, 6.5, SD 1.5 μm/h, respectively). The velocity of chondrocytes was increased by bFGF (10 ng/mL) ($p = 0.008$), but was not affected by HA. The enhancement of motility was seen in the presence of both HA (1 mg/mL) and bFGF (10 ng/mL) in chondrocytes up to 2.6 times. The stimulatory effect of bFGF (10 ng/mL) on chondrocyte migration was not seen after preincubation of bFGF with a polyclonal antibody raised against human bFGF (50 μg/mL). The spontaneous migration velocity of synovial cells was increased by HA (0.1 mg/mL, 1 mg/mL) ($p = 0.01$), but was not affected by bFGF or by the simultaneous administration of both (Figure 1).

Chemokinesis assays showed that bFGF significantly increased the directed migration of chondrocytes (1 ng/mL, 10 ng/mL) ($p = 0.008$), but did not influence that of synovial cells. On the contrary, HA significantly increased the directed migration of synovial cells (0.01 mg/ ml, 0.1 mg/mL, 1 mg/mL) ($p = 0.002$), but had no effect on chondrocytes (Figure 2).

Discussion

Our findings clearly showed that chondrocytes and synovial cells in monolayer culture migrate and they show chemokinesis to bFGF and HA, respectively. While most studies of the relationship between growth factors and the articular cartilage have focused on the metabolic effects on chondrocytes, less is known about their effects on reparative process, such as recruitment of precursor cells from synovial membrane and migration of chondrocytes in the regions adjacent to a defect. According to Hunziker and Rosenberg (1996), the release of growth factors at the articular cartilage caused migration of chondrogenic cells from the synovium. Some mitogens have been reported as a chemoattractant for chondrocytes, including BMP (Tanaka et al. 1995), insulin-like growth factor (Malemud 1993), and hepatocyte growth factor (Takebayashi et al. 1995) as well as fibronectin, type I, and type II collagens (Shimizu et al. 1997).

In our study, the optimum concentration of bFGF for chondrocyte migration was 10 ng/mL, which was much higher than that of cartilage matrix synthesis (0.4 ng/mL) (Kato 1992), and almost equal to that of cell proliferation (1 ng/mL, 10 ng/mL) (Matsusaki et al. 1998). In the arthritic joint, synoviocytes, chondrocytes, and extravasated inflammatory cells produced a high level of FGF and the

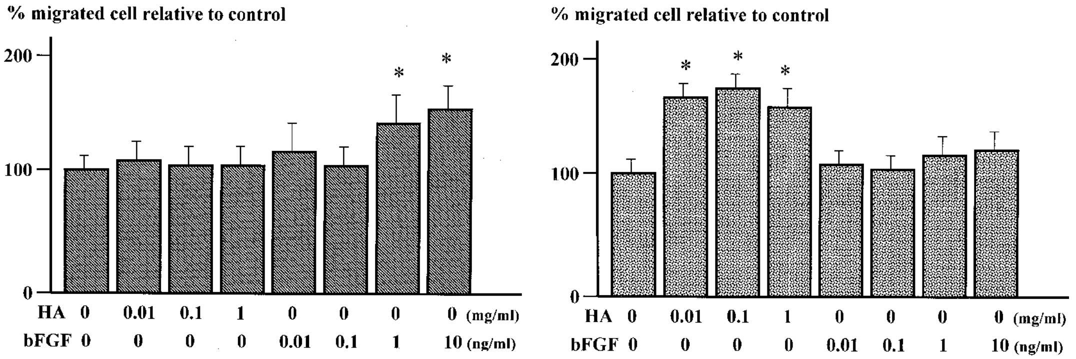

Figure 2. The chemokinetic response of chondrocytes (left) and synovial cells (right) to HA and bFGF at various concentrations. Results are expressed as the mean percentage of migrated cells per unit area (square millimeter) relative to the control group with standard deviation. Statistically significant differences between the control and experimental groups are indicated by * ($p < 0.01$).

release of FGF from the degraded cartilage matrix occurred (Sano et al. 1990). Under such conditions, we speculate that chondrocytes and chondrogenic cells undergo directed migration to the site of the cartilage lesion and initiate reparative processes. HA was also found in increased concentrations in inflamed tissues (Wells et al. 1992). Therefore, synovial cells are also capable of migration in the presence of large amounts of HA.

To induce healing in superficial defects of articular cartilage by simultaneously administering of both bFGF and HA is more likely to be effective, because bFGF has no negative effect on synovial cell motility, unlike the control in our study and HA is said to promote extracellular matrix synthesis and cell proliferation of chondrocytes in monolayer culture and 3-dimensional culture in collagen gel (Kawasaki et al. 1999). Onemust also study the temporal and spatial expressions of cytokines and their magnitude at the site of cartilage injury because mimicking and modifying the natural course of cytokine expression have great clinical implications.

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