Hyaluronate on heat shock protein and synovial cells in a canine model of osteoarthritis

Introduction/Summary

Intra-articular injection of hyaluronate (HA) is now widely used in the treatment of human arthropathies [1, 2, 3]. Using a model of arthritis induced in canine joints by anterior cruciate ligament transection (ACLT), we investigated the intra-articular effects of an HA preparation, HA84 (MW: 84 x 10^4), on the synovial tissue. Vacuolar degeneration of synovial cells was observed in the untreated animal model. This change was suppressed by the intra-articular treatment of HA84. When cells were exposed to an environmental insult, heat shock proteins were induced to suppress degeneration or cell death [4-6]. A slight expression of heat shock protein (Hsp72) was observed in the synovial cells of the untreated animal model. The expression of Hsp72 was enhanced by the treatment of HA84. These events suggest that the HA84 treatment protects or patronizes the cells by up-regulating the Hsp72 expression.

Methods/Results/Discussion

We used a slowly progressive model of early osteoarthritis or traumatic arthritis induced in canine knee joints by ACLT to examine the effects of HA84. HA84 (10 mg/ml) was prepared from rooster combs (Artz®, Seikagaku Corporation, Tokyo, Japan) as a highly pure, sterile, and pyrogen-free compound. The HA-salt was dissolved in phosphate buffered physiologic saline (PBS) to obtain a 1% solution with a protein content less than 0.1%. Electrophoresis detected no glycosaminoglycans other than HA.

The study included 13 male beagles, 5-8-month-old, weighing 7.0-9.0 kg. Three dogs were used as nonoperated normal controls (nonoperated noninjected group).

Arthritis was induced in both sterile joints by an ACLT in eight dogs. The whole procedure was carried out under general anesthesia (25 mg/kg body weight pentobarbitone).

Two of the eight operated dogs did not receive intra-articular injections, and were dissected 4 weeks after the ACLT (4w-ACLT group). The other six operated dogs received a single intra-articular injection of HA84 (50 µl/kg) into the left-knee joint (HA84 group) once a week for 4 weeks following the ACLT for a total trial of 5 weeks. Sterile PBS was injected intra-articularly into the right knee joints (PBS group) of the animals receiving HA84 by the same schedule as above. Sterile PBS was also injected intra-articularly into the four knee joints of two nonoperated animals (nonoperated, PBS-injected group) by the same schedule as described above.

Upon sacrifice, up to 30 mm in length of the synovium, was removed from the medial parapatellar region of synovium in the knee joints. The synovial tissues were fixed with 5% cetylpyridinium chloride–10% formalin for 24 h and embedded in OCT compound or paraffin. The tissue blocks were cut into 5 µm paraffin sections and stained with hematoxylin and eosin. Vacuolar degeneration of synovial cells was confined to the area of loose subsynovial connective tissue between the dense connective tissues.

Inflammation and vacuolar degenerative synovial cells, present 4 weeks after ACLT without injection (4w-ACLT group), was also observed in the PBS group [Fig. 1(b)]. Intra-articular injection of PBS caused no vacuolar degeneration of synovial cells, and inflammatory cell infiltration in the nonoperated joints (nonoperated, PBS-injected group, Table I). These events suggest that the inflammation and degeneration were caused by the
FIG. 1. Synovial tissues stained with hematoxylin and eosin in the (a) non-operated, non-injected group, (b) PBS group, (c) HA84 group. Arrows: vacuolar synovial cells (magnification: x400).

ACLT rather than by the PBS injection in the PBS group. There was less vacuolar degeneration of synovial cells in the HA84 group [Fig. 1(b), (c), Table I].

For the immunostaining of Hsp72 in the synovial cells, cryosections were incubated with anti-Hsp72 monoclonal antibody (Amersham, UK, 1:500) which reacts with 72 kDa heat shock protein. Moreover, to observe the localization of Hsp72 in the nucleus, the sections immunostained for Hsp72 were stained with propidium iodide that binds DNA. These sections were then observed with a confocal laser scanning microscope (Leica, Heidelberg, Germany).

Immunostainability for Hsp72 was faint in the nonoperated, noninjected group [Fig. 2(a)]. The incidence of the Hsp72 positive cells was slightly higher in the PBS group than in the nonoperated, noninjected group [Fig. 2(b); Table I]. Slight expression of Hsp72 was observed in the synovial cells of the 4w-ACLT group. There was no difference in the incidence of Hsp72 positive cells between the nonoperated, noninjected group and the nonoperated PBS injected group (Table I). This suggests that the slight expression of Hsp72 in the PBS group is caused by the ACLT rather than by the PBS injection. A strong expression of Hsp72 was observed in the HA84 group [Fig. 2(c)]. The incidence of the Hsp72 positive cells was higher in the HA84 group than in the nonoperated, noninjected group and the PBS group (Table I). In cells experiencing metabolic stress, newly synthesized proteins unable to properly fold become stably bound to Hsp72 [7]. Vass et al. have shown that Hsp72 accumulation is minimal in the hippocampal CA1 neurons, which die after brief ischemic episode, but is most pronounced in the dentate granule cells and CA3 neurons which are spared [8]. Heat shock proteins are essential to cells’ ability to survive environmental insult [6]. The vacuolar degeneration in the synovial cells was inversely correlated to the Hsp72 expression in the present study.

Hsp72 is transferred into the nucleus and nucleolus in heat shocked mammalian cells [9]. Lewis and Pelham have proposed a model in which ATP-driven cycles of binding and release of Hsp72 help to solubilize aggregates of proteins or ribonucleoproteins that form after heat shock [10]. In the present study, the incidence of nuclear Hsp72 positive cells were also higher in the HA84 group than in the PBS group [Fig. 2(b)–(e), Table I].

Most of heat shock protein-inducers, i.e., heat [10], sodium arsenite [11], hydrogen peroxide [12], and amino acid analogs [13] insult cells as stress factors. The HA treatment in the present study, however, enhanced the Hsp72 expression and suppressed the vacuolar degeneration in the synovial cells, suggesting that HA is not a stress factor.

Synovial cells may be exposed to several kinds of stress factors in the present arthritis model. The

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<th>Table I.</th>
<th>Histopathological examinations in synovium</th>
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<td>N−N</td>
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<td>N=6</td>
<td>N=4</td>
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<td>Percentage of Vacuolar synovial cells (s.e.m.)*</td>
<td>N.D.</td>
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<tr>
<td>Percentage of Hsp72 positive cells (s.e.m.)*</td>
<td>8.67†</td>
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<tr>
<td>Percentage of nuclear Hsp72(N) positive cells (s.e.m.)*</td>
<td>1.58‡</td>
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*Percentage incidence in 200 synovial lining cells; N−N: nonoperated, non-injected group; PBS−N: non-operated, PBS-injected group; 4w−ACLT: 4w-ACLT group; PBS: PBS group; HA84: HA84 group. N.D. not detectable; †P < 0.05 vs the HA84 group, ‡P < 0.01 vs the HA84 group, using Tukey’s multiple comparison test.
In conclusion, suppression of degeneration in the synovial cells was correlated with the up-regulation of Hsp72 in the present study, suggesting that HA84 treatment up-regulated the Hsp72 expression to suppress degeneration in cells under stress conditions. Further studies are required to verify the relationship between HA and Hsp72.

References


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