THE CARTILAGE REPARATIVE ACTIVITIES OF HIGH MOLECULAR WEIGHT HYALURONIC ACID MAY BE MEDIATED THROUGH ENHANCED CATABOLISM

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INTRODUCTION: Hyaluronic acid (HA) has been used as a therapeutic intervention in the treatment of osteoarthritits. We have reported that high molecular weight (800-kDa) HA is effective in blocking the catabolic action of fibronectin fragments (Fn-fs) on explant cultures of bovine cartilage and in an experimental in vivo model of rabbit knee joint damage. The protective mechanism appeared to involve the ability of the high molecular HA to block penetration of the Fn-f. Interestingly, HA was also effective in promoting restoration of proteoglycan (PG) in Fn-f treated and damaged human knee and bovine cartilage. We reported that this was associated with increased rates of PG synthesis. Our objective here was to explore this reparative mechanism in more detail. For this objective, the effects of different sized HAs on cartilage metabolism were compared.

METHODS

HAs from 6800 to 2 million daltons were kindly provided by Seikagaku Corp. of Japan. To test ability of HA to block Fn-f mediated cartilage PG depletion, bovine cartilage was cultured in 10% serum/DMEM and PG content of papain digests measured at various times. To test ability to block PG degradation, HA was added to cultures in DMEM alone and PG content of the media measured for each day of culture in order to derive rate constants. To test ability of HA to repair, bovine cartilage in 10% serum/DMEM was treated with 1 µM Fn-f for 7 days to deplete half of the matrix PG. Fn-f was removed and HA added at 1 mg/ml. PG content was analyzed each week. Rates of incorporation of 35-S sulfate were used as PG synthesis rates. Rabbit antibodies to human VDIPEN and NITEGE neoepitopes were kindly provided by D. Visco of Merck Research Laboratories and sheep anti-human MMP-3 polyclonal antibodies were provided by DuPont Merck Research Laboratories.

RESULTS

Higher molecular weight HA forms were the most effective in terms of blocking decreases in PG content in Fn-f treated cartilage and restoring PG in Fn-f damaged cartilage. Bovine cartilage explant cultures were incubated with 1 mg/ml of HAs and PG content measured at days 7, 14, 21. HA of 60-kDa and lower decreased cartilage PG content. The smaller HAs were ineffective in restoring PG in Fn-f damaged cartilage, while HA 60, 250 and 800-kDa promoted restoration of PG as shown in Fig 1 with HA 60-kDa the slowest. HA 800-kDa had the greatest cartilage protection properties.

The enhanced PG synthesis in cartilage associated with HA treatment was associated with decreased release of endogenous IGF-1 into the media and enhanced retention within the cartilage matrix of exogenously added rhodamine IGF-1 – Bovine cartilage in 10% serum/DMEM was incubated with 1 mg/ml HA 800-kDa and media assayed for IGF-1 at various times. IGF-1 release compared to control was markedly decreased (Fig 2) even in the presence of the Fn-f, which by itself enhanced release. Addition of rhodamine labeled IGF-1 to cartilage followed by addition of HA 800-kDa and fluorescence microscopy showed that HA enhanced retention of IGF-1 and decreased the ability of Fn-f to enhance release. However, HA did not block release of endogenously labeled protein. Thus, HA appears to enhance activity of IGF-1 but does not do this through a general nonspecific effect of trapping proteins within the matrix.

The various HA forms also enhanced expression of the NITEGE and VDIPEN neoepitopes in bovine cartilage by 7 days, neoepitopes that are characteristic of physiologically relevant PG degradation. Fig 3 shows enhanced NITEGE in HA-250-kD treated cartilage. The HA forms also enhanced expression of MMP-3 after an extended period and the smaller HA forms also enhanced rates of PG degradation in serum free cultures by up to 40% over control levels. HA800-kDa was the least active. Since we have reported that HA-800-kDa did not enhance these neoepitopes or MMP-3 in human knee tissue, we assume these differences may be due to innate differences in kinetics of or catabolic pathways in these species.

DISCUSSION

We have shown that HA enhances retention of matrix IGF-1, with subsequently enhanced PG synthesis, and also induces a low grade of matrix turnover. We hypothesize that slightly enhanced proteolytic activity activates the IGF-1 system or other factors perhaps through enhanced turnover of other matrix molecules or through specific degradation of IGF-1 binding proteins that otherwise trap IGF-1 within the matrix. It is also possible that the slightly enhanced proteolysis observed here is secondary and that HA mechanically perturbs the matrix and enhances activation of IGF-1 or other factors. Nonetheless, regardless of the model, the variable effects of high molecular weight HA in clinical trials may be due, in part, to variability in retention of IGF-1 or other factors or reduced sensitivity to these factors in aging cartilage.