· HYALURONAN SUPPRESSES IL-ß-INDUCED METALLOPROTEINASE ACTIVITY FROM SYNOVIAL LINING CELLS IN PATIENTS WITH OSTEOARTHRITIS

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INTRODUCTION:

Viscosupplementation using hyaluronans (HAs) is approved for treating pain due to osteoarthritis (OA). One possible mechanism is the reduction in expression of metalloproteinases (MMPs), cartilage-destroying enzymes that are produced in response to proinflammatory cytokines including interleukin-1ß (IL-1ß). We hypothesized that the HAs reduced IL-1ß-induced MMPs secreted by synovial lining cells in OA patients.

METHODS:

Synovial biopsies were obtained from patients having total knee replacement for OA, Kellgren-Lawrence Grade IV. All experimental procedures were approved by the Institutional Review Board for Human Research at our institution. The tissues were taken from the suprapatellar pouch and the fat pad using the full bite of an arthroscopic basket punch. Only relatively flat areas were biopsied to minimize variations in the number of synovial lining cells attributable to irregularities in the synovial surface. In preliminary studies, we established that the biopsies averaged 20 mg (0.12 cm^2) ±15%. Twelve biopsies were obtained from each patient.

Three FDA-approved HAs were obtained commercially; Hyalgan (HY, 0.5-0.7 MDa); Supartz (SU, 0.6-1.2 MDa); Synvisc (SY, 6.0 MDa). A non-approved commercially available HA (LifeCore, LI, 0.2 MDa) was also studied. The HAs were supplied in PBS and were transferred to Neuman-Tytell media (NTM) by dialysis, performed at 5°C for one week, except LI which was supplied as a powder and directly dissolved in NTM. All HA solutions were adjusted to a concentration of 8 mg/mL.

Immediately after the biopsies were done, the tissues were washed 4 times in NTM and then incubated at 37°C for 24 hours in 200 µL NTM containing various combinations of IL-1ß (100 pg/mL) and HAs. The MMP activity of the supernatant (principally MMP-1 and MMP-3) was then measured using collagen film as a substrate, after MMP activation by 1 mM p-amino phenylmercuric acetate; further details are given elsewhere (1).

RESULTS:

In the first experiment, for each of 5 patients, the mean of three independent measurements of MMP activity from 3 biopsies was used to characterize the level of constitutive MMP production, the mean of three additional biopsies was used to characterize the response to IL-1ß, and the remaining six biopsies were divided and used to assess the ability of LI and SU to affect the IL-1ß-induced MMP production. When the results were averaged over the 5 patients (grand means computed from the individual patient means) we found that LI had no effect on stimulated MMP levels, but that SU reduced MMP activity to baseline levels (Figure 1). The experiment was repeated with five additional patients, and we found that HY had no effect on the ability of IL-1ß to stimulate MMP production, but SY reduced MMP levels to baseline levels (Figure 2). None of the HAs tested affected MMP production by synovial tissue from OA patients in the absence of IL-1ß (Figure 3).

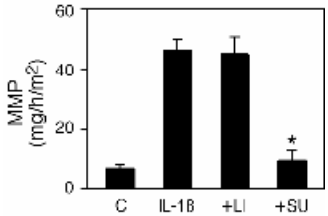

*Figure 1. Effect of Lifecore (LI) and Supartz (SU) on IL-1ß-induced MMP production by synovial tissue from patients with Grade IV OA. When synovial biopsies from patients with OA were cultured in the presence of IL-1ß (100 pg/ml) for 24 hours, the amount of MMP activity in the supernatant was increased compared with the control (C) synovial biopsies (no IL-1ß). Addition of IL-1ß and LI (8 mg/mL) together (+LI) had no effect on IL-1ß-induced MMP production, but addition of IL-1ß and SU (8 mg/mL) together (+SU) reduced stimulated secretion of MMPs to baseline. Three measurements per condition per patient. N=5 patients. The individual means for each patient were used to compute the overall patient means (±SE). *P<0.05 compared with IL-1ß alone.*

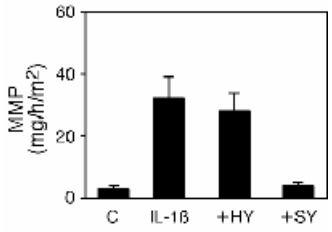

*Figure 2. Effect of Hyalgan (HY) and Synvisc (SY) on IL-1ß-induced MMP production by synovial tissue from patients with Grade IV OA. When synovial biopsies from patients with OA were cultured in the presence of IL-1ß (100 pg/mL) for 24 hours, the amount of MMP activity in the supernatant was increased compared with the control (C) synovial biopsies (no IL-1ß). Addition of IL-1ß and HY (8 mg/mL) together (+HY) had no effect on IL-1ß-induced MMP production, but addition of IL-1ß and SY (8 mg/mL) together (+SY) reduced stimulated secretion of MMPs to baseline. Three measurements per condition per patient. N=5 patients. The individual means for each patient were used to compute the overall patient means (±SE). *P<0.05 compared with IL-1ß alone, paired t test.*

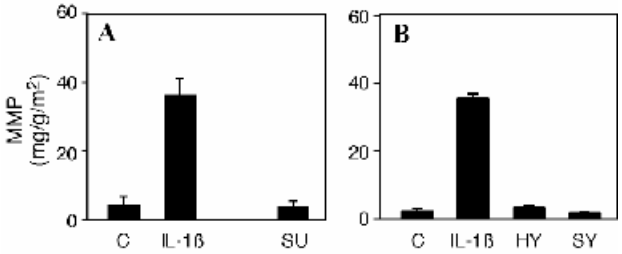

Figure 3. Supartz (SU), Hyalgan (HY), and Synvisc (SY) had no effect on MMP production by synovia from patients with OA in the absence of stimulation by IL-1ß. Three measurements per condition per patient. The individual means from each patient were used to compute the overall patient means (±SE). A, N=3 patients; B, N=3 patients.

DISCUSSION:

HAs blocked IL-1ß-induced MMP activity produced by synovial lining cells via a process that may have depended on HA molecular weight, because inhibition occurred only with the HAs having the higher molecular weights. It is not possible to reach conclusions regarding the relative abilities of the HAs to inhibit cytokine-induced MMP activity at the concentrations approved for clinical use (10 mg/mL, except 8 mg/mL for Synvisc). Even so, the results established that HAs can inhibit cytokine-induced MMP activity, as hypothesized, suggesting that the mode of action of HA might be due to reduced MMP activity, with concomitant reduction in the pain caused by inflammation secondary to the destruction of cartilage by MMPs.

REFERENCES:

1. Kolomytkin OV, Marino AA, Waddell DD, Mathis JM, Wolf RE, Sadasivan KK, Albright JA. Am J Physiol 282:C1254-C1260, 2002.

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