Agents Actions 37 (1992)

0065-4299/92/020155-02 $ 1.50+0.20/0

The effect of hyaluronan on interleukin-1α-induced prostaglandin E_2 production in human osteoarthritic synovial cells

T. Yasui, M. Akatsuka, K. Tobetto, M. Hayaishi[1], T. Ando

Division of Biochemical Pharmacology, Research Laboratories, Maruho Co., LTD., 1-8-23 Oyodo Naka, Kita-ku, Osaka 531, Japan; [1] Hayaishi Hospital, Osaka, Japan

Abstract

An *in vitro* study on the effects of hyaluronan (HA) on interleukin-1α-induced prostaglandin E_2 (PGE_2) production in human osteoarthritic synovial cells indicated that PGE_2 induction was suppressed by HA in a dose- and molecular weight-dependent manner.

Introduction

Although osteoarthritis (OA) is primarily a disorder of the cartilage, secondarily induced synovitis is caused by the products released during the process of cartilage breakdown. IL-1 released from inflamed tissues activates synoviocytes to produce subsequently PGE_2 [1]. PGE_2 potentiates the nociceptive activity of bradykinin and amplifies pain perception [2]. Recent clinical trials for OA using intra-articular injection of hyaluronan (HA) with a high molecular weight (M_r) of 6.3×10^5 have revealed its analgesic action [3] and a reduction of PGE_2 levels in human synovial fluids [4]. These results suggest that the analgesic action of HA may be attributed to the reduction of synovial PGE_2 levels. To examine if HA actually suppressed PGE_2 production at the cellular level, the effects of HA with different M_r's on IL-1α-induced PGE_2 production by human OA synovial cells were studied.

Materials and methods

HAs of various M_r's (viscosity-average $M_r = 2.0 \times 10^6$, 1.6×10^6, 1.0×10^6, 0.3×10^6; gifts of Shiseido Pharm. Res. Lab., Japan) were purified (protein content less than 0.1%) from culture broths of *Streptococcus zooepidemicus* and were then tested to be endotoxin-free. Synovial cells, prepared by enzymatic digestion of synovium from an OA patient [1], were cultured in DMEM containing 20% FCS at 37 °C in an atmosphere with 5% CO_2. Monitored under phase-contrast microscopy, fibroblasts were removed by incubating the cells in 0.125% trypsin–0.01% EDTA [5]. Trypsin-resistant adherent cells were further cultured in the above medium prior to harvesting with a rubber policeman. The cells were then seeded in 6-well plates at 2.5×10^5 cells/well and cultured at 37 °C for 24 h, and washed with DMEM before stimulation by 2.25 ml of the fresh medium (10% FCS) containing 10 U/ml of human recombinant IL-1α (Genzyme) in the presence or absence of HA. Various M_r's of HA were employed at concentrations of 0.25, 0.5, or 1.0 mg/ml. After 45 h of incubation, PGE_2 in the medium was determined. For PGE_2 assay, PGE_1 (Ono Pharm., Japan) was added to 1.5 ml of the medium and used as an internal standard. According to previously established methods, PGs were extracted [6] and derivatized with panacyl bromide (Polyscience, USA) [7]. The PG panacyl esters, which were analyzed using the

HPLC with a normal phase column (PGpak B, Jasco, Japan) with isooctane–ethyl-acetate–ethanol–acetic-acid (69:25:6:2) as the mobile phase, were quantitatively measured by the fluorescence method (E_x; 249 nm, E_m; 470 nm). This method produced a linear correlation coefficent exceeding 0.999 over a PGE_2 concentration range of 0–200 ng, and the recovery rate for PGE_2 was 95.4%.

Results

Trypsin-resistant cells indicated either an oval or rhombic shape. PGE_2 production in these cells was enhanced by about 16-fold compared to the spontaneous release on exposure to 10 U/ml of IL-1α. HA, at 0.5 and 1.0 mg/ml, reduced IL-1α-induced PGE_2 production in a dose- and M_r-dependent manner (Fig. 1). At the lowest dose of 0.25 mg/ml, the inhibitory effect of HA was insignificantly low (except with M_r of 2.0×10^6). On verifying the inhibitory effects at the highest dose (1.0 mg/ml), significant effects with all M_r's used were achieved. Further, results of the maximum inhibition (46.2%) on induced PGE_2 production (1.0 mg/ml, 2.0×10^6 M_r) indicated statistical significance when compared to other preparations with lower M_r's at the same dose. HA did not affect the cell density and viability of the cells assessed by trypan blue exclusion after 48 h incubation.

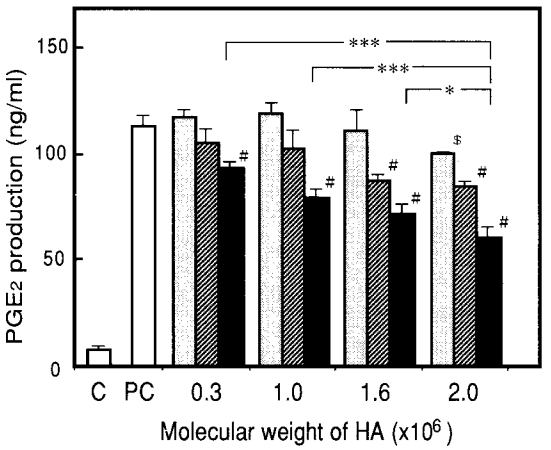

Figure 1
Subcultured cells were stimulated in a medium with (positive control, $n = 4$: PC) or without (control, $n = 4$: C) 10 U/ml of IL-1α. PGE_2 levels in each medium were assayed after 45 h incubation. Results represent mean ± SD of 3 (0.25 mg/ml of HA: dotted column) or 4 (0.5 mg/ml: hatched column and 1.0 mg/ml: closed column) individual wells of a culture. Significant differences, evaluated by the unpaired Student's t-test, are indicated by the following symbols: $^{\#}p < 0.001$; $^{\$}p < 0.01$ (versus the positive control); $^{*}p < 0.05$; $^{***}p < 0.001$ (data at 1.0 mg/ml with 2.0×10^6 M_r versus other results with lower M_r's).

Discussion

Synovial tissues are composed of heterogeneous cells. Macrophage-like synoviocytes are responsible for inducing PGE_2 production in cloning studies [8]. Thus, we examined the effects of HA on macrophage-like synovial cells, which potently produce PGE_2 on stimulation with IL-1α. When tested at three concentrations and various M_r's in our present study, HA reduced IL-1α-induced PGE_2 production in the following manner: the higher the M_r and dosage of HA, the more potent were the inhibitory effects on PGE_2 induction. These findings could account for the mechanism of PGE_2 reduction in synovial fluids on intra-articular administration of HA [4]. As such, these findings suggest that a higher M_r of HA may be more effective in relieving pain related to arthritis. Further studies to elucidate the site and mechanism of HA are warranted.

Received 23 April 1992; accepted by M. J. Parnham
3 May 1992

References

[1] J.-M. Dayer, B. de Rochemonteix, B. Burrus, S. Demczuk and C. A. Dinarello, *Human recombinant interleukin 1 stimulates collagenase and prostaglandin E_2 production by human synovial cells.* J. Clin. Invest. *77,* 645–648 (1986).

[2] S. H. Ferreria, S. Moncada and J. R. Vane, *Potentiation of prostaglandins of the nociceptive activity of bradykinin in the dog knee joint.* Br. J. Pharmacol. *50,* 461 (1974).

[3] A. S. J. Dixon, R. K. Jacoby, H. Berry and E. B. D. Hamilton, *Clinical trial of intra-articular injection of sodium hyaluronate in patients with osteoarthritis of the knee.* Curr. Med. Res. Opin. *11,* 205–213 (1988).

[4] L. Punzi, F. Schiavon, F. Cavasin, R. Ramonda, P. F. Gambari and S. Todesco, *The influence of intra-articular hyaluronic acid on PGE_2 and cAMP of synovial fluid.* Clin. Exp. Rheum. *7,* 247–250 (1989).

[5] S. Saito, P. Nagan, M. Saito, R. Lanese, J. Shanfeld and Z. Davidvitch, *Interactive effects between cytokines on PGE production by human periodontal ligament fibroblasts in vitro.* J. Dent. Res. *69,* 1456–1462 (1990).

[6] W. S. Powell, *Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica.* Prostaglandins *20,* 947–957 (1980).

[7] T. A. Stein, L. Angus, E. Borrero, L. J. Auguste and L. Wise, *Picogram measurement of prostaglandin E_2 synthesis by gastric nucosa by high-performance liquid chromatography.* J. Chromatogr. *385,* 377–382 (1987).

[8] M. Sasano, M. Goto and K. Nishioka, *Production of prostaglandin E_2 induced by histamine by cloned rheumatoid synovial cells.* Ann. Rheum. Dis. *49,* 504–506 (1990).