CONCISE REPORT

Histamine stimulates matrix metalloproteinase-3 and -13 production by human articular chondrocytes in vitro

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Objectives: To determine the effects of histamine on matrix metalloproteinase (MMP) production by human articular chondrocytes (HAC) in vitro.

Methods: Conditioned culture medium from HAC cultures incubated with and without 20 μ M histamine was assayed by enzyme linked immunosorbent assay (ELISA) for MMP-1, MMP-8, MMP-13 (collagenases 1, 2, and 3, respectively) and MMP-3 (stromelysin). Monolayer cultures of HAC were also immunostained for MMP-13 and MMP-3.

Results: The HAC cultures showed a significant increase in MMP-13 and MMP-3 production (2.2- and 1.9-fold, respectively) after treatment with 20 μ M histamine for 24 hours, but MMP-1 and MMP-8 were unaffected. All cultures showed MMP-13 and MMP-3 detectable by immunolocalisation. MMP-3 was the more prominent enzyme as shown by both ELISA and immunolocalisation techniques.

Conclusions: Histamine exposure increased both MMP-13 and MMP-3 production by HAC in vitro, thereby suggesting a pathophysiological role in the chondrocytic phenotype associated with degenerative changes in osteoarthritis.

Degeneration and loss of articular cartilage are characteristic features of osteoarthritis (OA). Fibrillations of the articular surface, matrix depletion, cell clusters, and changes in matrix composition reflect the aberrant behaviour of resident chondrocytes, but as yet the pathogenesis of OA remains unclear. The matrix metalloproteinases (MMPs) and proinflammatory cytokines are considered important for the chondrolytic processes that contribute to the degenerative changes in OA cartilage (reviewed by Pelletier *et al*¹). Measurable levels of histamine are present in a significant proportion of synovial fluids from OA joints^{2,3}; and mast cells, which are the prime source of histamine, are found in increased numbers in arthritic joints.⁴ Histamine has a recognised role in allergic and inflammatory reactions, and is reported to affect various cell types, such as macrophages, epithelial cells, eosinophils, and various subsets of T cells.^{5,6} We have previously shown that receptors for histamine, described as H₁ and H₂, are expressed by human articular chondrocytes (HAC); stimulation of the H₁ receptors results in increased prostaglandin E₂ (PGE₂) production, while stimulation of the H₂ receptors results in increased intracellular cyclic AMP.⁷

Chondrocytes have been shown to produce three different collagenases (MMP-1, MMP-8, and MMP-13) and stromelysin 1 (MMP-3) in situ, enzymes considered crucial for collagen and aggrecan degradation.^{8,9} This study therefore examined whether histamine modulates the production of these MMPs by HAC in vitro.

MATERIALS AND METHODS

Cell cultures

HAC were isolated from cartilage from osteoarthritic tissues removed at joint replacement surgery as described.⁸ Patient consent and ethical approval was obtained for all the tissues used in this study. All cultures were of a chondrocytic phenotype as shown by positive S100 and type II collagen immunostaining as demonstrated previously.¹⁰ Cells from four different cartilage samples were grown to confluence in 12 well culture dishes in Dulbecco's modified Eagle's medium (DMEM) + 10% fetal calf serum (FCS). Triplicate wells of first or second passage HAC were treated with either DMEM + 2% FCS alone (control), or DMEM + 2% FCS and 20 μ M or 100 μ M histamine. All cell culture experiments were incubated for 24 hours at 37°C, the conditioned medium harvested, centrifuged, and assayed for MMP production by enzyme linked immunosorbent assay (ELISA). In addition, mepyramine and ranitidine, specific H₁ and H₂ histamine receptor antagonists, respectively, were added to the cells at a concentration of 20 μ mol/l, together with histamine. HAC cells were also incubated with indometacin (14 μ mol/l) to inhibit prostaglandin synthesis. Conditioned culture media from these experiments were assayed for MMP-3 and MMP-13.

After each experiment, cells were fixed with 70% ethanol, stained with toluidine blue, covered with Immunomount mounting medium (Shandon, UK) and a cover glass, and counted using an Olympus inverted microscope. Twelve 1 mm² fields were counted for each plate and the number of cells per well calculated.

MMP measurement

MMP-13 and MMP-8 were assayed in the conditioned culture media using ELISA kits from Amersham UK Ltd. For both these enzymes the pro- and active forms are assayed; proMMP-1 was assayed using an ELISA kit from the Binding Site, Birmingham, UK; and MMP-3 was measured by an in-house sandwich ELISA.¹¹ Results were calculated as ng or pg MMP/10⁶ cells/24 h.

Immunolocalisation

HAC were grown in four-chamber culture slides (Nunc, Gibco, UK) and incubated as described above with or without histamine. At 20 hours 5 μ M monensin, which inhibits the secretion of newly synthesised products, was added for four hours followed by fixation in 70% ethanol. Cells were subsequently air dried and stored at 4°C. After rehydration in Tris buffered saline (TBS) cells were immunostained using sheep primary antibodies to MMP-13 (a generous gift from Dr

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme linked immunosorbent assay; FCS, fetal calf serum; HAC, human articular chondrocytes; IL, interleukin; MMP, matrix metalloproteinase; OA, osteoarthritis; PGE₂, prostaglandin E₂; TBS, Tris buffered saline; TNF α , tumour necrosis factor α .

Figure 1 Effect of histamine on the production of MMP-13 and MMP-3 by HAC in culture over 24 hours. (A) Production of MMP-13 by four different HAC cultures without (open bars) and with (grey bars) 20 μ M histamine treatment. The filled bars for two of the cultures represent data at 100 μ M histamine treatment. (B) Mean data for the production of MMP-13 by six different HAC cultures with and without 20 μ M histamine treatment (* $p=0.005$, Student's t test). (C) MMP-3 produced by four different HAC cultures without (open bars) and with (grey bars) 20 μ M histamine treatment. (D) Mean data for the production of MMP-3 by six different HAC cultures with and without 20 μ M histamine (* $p=0.02$, Student's t test).

D Taylor, The Binding Site, Birmingham, UK) and MMP-3 (Biogenesis, Poole, UK) diluted 1:100 and 1:250 in TBS, respectively, and FITC-labelled rabbit anti-sheep secondary antibodies (DAKO, UK). Cells were viewed with a Zeiss Photomicroscope III and photographed using Kodak Tmax 100pro black and white negative film.

RESULTS

MMP-13 was produced by all the chondrocyte cultures examined (fig 1A). Histamine (20 μ M) proved as effective at stimulating MMP-13 production as 100 μ M histamine for HAC1 and HAC2 over 24 hours (fig 1A). The mean (SD) control value calculated from six different cultures was 267 (63) pg MMP-13/10⁶ cells/24 h compared with the 20 μ M histamine treated value of 596 (121) pg MMP-13/10⁶ cells/24 h (fig 1B). Thus

MMP-13 was stimulated approximately 2.2-fold by the addition of 20 μ M histamine.

MMP-3 production by the HAC cultures was also increased after treatment with 20 μ M histamine (fig 1C). The mean (SD) control value for MMP-3 was 274 (120) ng MMP-3/10⁶ cells/24 h compared with the 20 μ M histamine-treated value of 523 (141) ng MMP-3/10⁶ cells/24 h (fig 1D), representing an increase of 1.9-fold.

Although MMP-8 was not detected in the HAC cultures examined, either with or without histamine, MMP-1 production was approximately 40.8 (9.3) ng MMP-1/10⁶ cells/24 h ($n=4$). Histamine, however, had no effect on the level of MMP-1 production.

Table 1 shows the effects of specific H₁ or H₂ receptor antagonists on the histamine treated cells. Histamine stimulation of MMP-3 and MMP-13 production was blocked by the H₁

Table 1 Effect of histamine H₁ and H₂ receptor antagonists, and indometacin, on the histamine stimulation of MMP-3 and MMP-13 production by HAC

	MMP-3 (ng/10 ⁶ cells/ 24 h)	MMP-13 (pg/10 ⁶ cells/ 24 h)
Experiment 1. (HAC 2)		
Control	444.3 (47.8)	343.9(6.5)
+ histamine (20 µM)	694.2 (62.1)	1049.0 (31.2)
+ histamine + mepyramine (20 µM)	223.8 (29.5)	529.2(15.8)
+ histamine + ranitidine (20 µM)	430.7 (41.6)	829.1(24.7)
Experiment 2. (HAC 5)		
Control	572.3 (34.8)	190.9(11.7)
+ histamine (20 µM)	962.4 (56.9)	420.4(37.8)
+ indometacin (14 µM)	609.0 (56.4)	200.8(19.7)
+ histamine + indometacin	670.1 (45.2)	401.9(41.7)

antagonist, mepyramine, and reduced by the H₂ antagonist, ranitidine. Table 1 also shows that the addition of indometacin to HAC cultures treated with histamine abolished the stimulation of MMP-3 production, whereas that for MMP-13 remained unaffected.

Both enzymes were immunolocalised to a proportion of cells in each of three different HAC cell cultures. The immunostaining for MMP-3 was much more intense than that for MMP-13 (fig 2), reflecting ELISA results for secreted enzymes measured as ng and pg/10⁶ cells /24 h, respectively.

DISCUSSION

Recent studies of OA cartilage have identified both messenger RNA (mRNA) and the protein for specific MMPs as well as a collagenase mediated type II collagen degradation product, suggesting that MMPs contribute to the intrinsic chondrocyte mediated degenerative changes of the cartilage matrix in OA.^{8-12,13} As yet the factors responsible for their expression remain uncertain, although the proinflammatory cytokines interleukin 1 (IL1) and tumour necrosis factor α (TNF α) have been implicated.¹⁻⁸ The increased levels of histamine found in OA synovial fluids³ have suggested a role for this mediator in the pathophysiology of this disease. Evidence presented here shows that histamine up regulates both MMP-13 and MMP-3 production by chondrocytes. Both these MMPs are important in the degradation of articular cartilage; MMP-13 can degrade collagen type II, and MMP-3 can degrade proteoglycan and collagen types IX and XI, and activate procollagenase-1.¹⁴ Earlier studies have shown that chondrocytes possess both H₁ and H₂ histamine receptors,⁷ and because mepyramine prevented the up regulation of both MMP-3 and MMP-13 by histamine the data suggest that this is mediated through the H₁ rather than the H₂ pathway. Histamine is known to stimulate PGE production by HAC,⁷ and this may also contribute to the regulation of MMP production; a reduction in PGE apparently reducing MMP-3 but not MMP-13 expression. Although the concentrations of histamine used for in vitro studies^{7,15,16} are higher than those reported in OA synovial fluids,¹⁷ as yet there is no information on localised tissue concentrations of released histamine.

Both immunolocalisation and ELISA data suggest that MMP-3 production by HAC in vitro is quantitatively more prominent than that of MMP-13. However, in vivo, the production of these MMPs by chondrocytes in OA cartilage has been shown to vary with zone and disease progression.^{8-12,13} The localisation results demonstrate that only a proportion of the HAC in culture were producing

Figure 2 Immunolocalisation of MMP-13 and MMP-3 in HAC cultures. (A) Immunolocalisation of MMP-13 visualised with FITC. Note only a proportion of cells (HAC2) are positively stained. (B) Immunolocalisation of MMP-3 visualised with FITC. Not all cells (HAC2) are positive, but note the higher intensity of staining compared with MMP-13 in (A). (C) Negative control for (A) and (B). Bar =20 µm for all micrographs.

enzyme, an observation which may reflect preferential expression of specific receptors or their position in the cell cycle.

Zenmyo *et al* reported a modest histamine H₁ receptor-induced increase of mRNA for MMP-1 and its protein by immunohistochemistry, by rheumatoid synovial fibroblasts over 24 hours in vitro.¹⁵ However, the HAC experiments reported here have shown that production of both MMP-1 and MMP-8 by HAC was not affected by histamine treatment after 24 hours, confirming the differential regulation of collagenases 1, 2, and 3 in chondrocytes as previously demonstrated both in vitro and in situ.⁸⁻¹⁰ Because histamine-induced increases in cAMP, PGE₂, intracellular Ca²⁺, and inositol triphosphate accumulation, as well as H₁ receptor expression, were all recorded within minutes of exposure to histamine,^{7,15}

a 24 hour culture period with added histamine would seem to be appropriate for an appraisal of MMP production.

We have previously shown that IL1 and TNF α are synthesised by HAC in OA cartilage, potentially providing autocrine factors for MMP expression, PGE production, and the inhibition of matrix synthesis.⁸ Interestingly, histamine is reported to be a modulator of cytokine production, especially the induction of the IL18 cytokine cascade, which in turn may play a part in the stimulation of histamine synthesis.¹⁸ There are several reports of raised histamine levels in the synovial fluids of OA joints,^{2,3,17} presumably provided by mast cells of OA synovial tissue⁴ or possibly by activated chondrocytes,¹⁹ although its unequivocal demonstration within OA cartilage requires further study. The histamine induced changes in the HAC phenotype reported here suggest that histamine:chondrocyte interactions may well contribute to the degenerative changes associated with OA cartilage.²⁰

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