Effects of the antirheumatic remedy Hox alpha - a new stinging nettle leaf extract - on matrix metalloproteinases in human chondrocytes *in vitro*

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Summary. Inflammatory joint diseases are characterized by enhanced extracellular matrix degradation which is predominantly mediated by cytokine-stimulated upregulation of matrix metalloproteinase (MMP) expression. Besides tumour necrosis factor- (TNF-), Interleukin-1ß (IL-1ß) produced by articular chondrocytes and synovial macrophages, is the most important cytokine stimulating MMP expression under inflammatory conditions. Blockade of these two cytokines and their downstream effectors are suitable molecular targets of antirheumatic therapy. Hox alpha is a novel stinging nettle (*Urtica dioica/Urtica urens*) leaf extract used for treatment of rheumatic diseases.

The aim of the present study was to clarify the effects of Hox alpha and the monosubstance 13-HOTrE (13-Hydroxyoctadecatrienic acid) on the expression of matrix metalloproteinase-1, -3 and -9 proteins (MMP-1, -3, -9). Human chondrocytes were cultured on collagen type-II-coated petri dishes, exposed to IL-1ß and treated with or without Hox alpha and 13-HOTrE. A close analysis by immunofluorescence microscopy and western blot analysis showed that Hox alpha and 13-HOTrE significantly suppressed IL-1ß-induced expression of matrix metalloproteinase-1, -3 and –9 proteins on the chondrocytes *in vitro*.

The potential of Hox alpha and 13-HOTrE to suppress the expression of matrix metalloproteinases may explain the clinical efficacy of stinging nettle leaf extracts in treatment of rheumatoid arthritis. These results suggest that the monosubstance 13-HOTrE is one of the more active antiinflammatory substances in Hox alpha and that Hox alpha may be a promising remedy for therapy of inflammatory joint diseases.

Key words: Hox alpha, 13-HOTrE; Interleukin-1ß; Matrix metalloproteinase, Chondrocyte

Introduction

Articular cartilages contain chondrocytes embedded in a well-developed matrix composed of collagen type-II fibrils and proteoglycans. Enzymatic degradation of joint cartilage is one of the most important events in osteoarthritic and rheumatoid arthritic joint destruction. In arthritic cartilage, chondrocytes, synoviocytes and infiltrating macrophages produce proinflammatory cytokines such as interleukin-1ß (IL-1ß) and tumour necrosis factor- (TNF-), which promote the cartilage cells to produce destructive enzymes, such as matrix metalloproteinases (MMPs), that degrade the cartilage-specific extracellular matrix (Gowen et al., 1984; Feldmann et al., 1996b; Martel-Pelletier, 1998). Several studies have reported that in rheumatoid arthritis and osteoarthritis patients IL-1ß and TNF- are key proinflammatory cytokines in mediating cartilage degradation in these processes (Gowen et al., 1984; Brennan et al., 1992; Feldmann et al., 1996b; Martel-Pelletier, 1998).

MMPs belong to an enzyme family of zinc-dependent endopeptidases involved in the physiological turnover and pathophysiological breakdown of extracellular matrix in connective tissues such as wound healing, angiogenesis, chondrogenesis, tumour metastasis and arthritis (Martel-Pelletier et al., 1994; Cawston, 1996; Yong et al., 1998; Murphy and Gavrilovic 1999; Schulze-Tanzil et al., 2001). Indeed, MMP-1, MMP-3 and MMP-9 are reported to be increased in osteoarthritic cartilage and rheumatoid arthritis (Freemont et al., 1997; Horton et al., 1998; Saito et al., 1998). MMPs are expressed as inactive forms which, after proteolytic activation, are able to degrade extracellular cartilage matrix (ECM) components including proteoglycans, fibronectin, laminin and the collagen network (Fosang et al., 1991; Saito et al., 1998). Several classes of MMPs exist, including collagenases (MMP-1, -8 and -13), the gelatinases (MMP-2 and -9) and stromelysins (MMP-3, -10, -11) (Suzuki et al., 1990; Ogata et al., 1992; Cawston, 1996;

Knäuper et al., 1996; Murphy and Knäuper, 1997). Especially, MMP-13 seems to play a pivotal role in type-II collagen degradation observed in arthritis (Billinghurst et al., 2000; Neuhold et al., 2001). These enzymes are inhibited by tissue inhibitors of metalloproteinases (TIMPS) (Martel-Pelletier et al., 1994; Will et al., 1996). In the last few years other matrix metalloprotease-related enzymes have been detected in cartilage and have focused much interest; e.g. aggrecanases (Arner et al., 1998, 1999; Abbaszade et al., 1999). Aggrecanases cleave aggrecan, a major extracellular matrix component, and seem to be primarily responsible for extracellular matrix proteoglycan degradation, since aggrecan is one of the first matrix components to undergo measurable loss in arthritic disease (Tortorella et al., 2000). Moreover, members of the ADAM family of proteinases have been found in cartilage, but their particular substrates and functions remain obscure at the moment (McKie et al., 1997; Chubinskaya et al., 1998, 2001). Therefore, inhibition of enzymatic activity of MMPs or suppression of MMP expression are realistic targets of antirheumatic therapy.

Stinging nettle (*Urtica dioica*/*Urtica urens*) leaf extracts are registered in Germany for adjuvant therapy of rheumatic diseases. The efficacy data are summarized in so-called monographs (BGA, 1990, ESCOP, 1997) and have been proven by several post-marketing surveillance studies (Ramm and Hansen, 1995, 1996; Wolf, 1998). In the past, it has been reported that leaf extracts from stinging nettle suppressed the expression of different cytokines in immune cells *in vitro* (Obertreis et al., 1996; Klingelhoefer et al., 1999). Furthermore, the transcription factor NF- B has been identified as a potential molecular target of antiinflammatory stinging nettle leaf extracts (Riehemann et al., 1999).

The present study was designed to determine the effects of Hox alpha on the expression of MMPs in IL-1ß-stimulated or unstimulated human chondrocytes. We used human chondrocytes for our experiments and studied the effects of Hox alpha and the monosubstance 13-HOTrE on MMP expression *in vitro* by immunofluorescence microscopy and western blot analysis. In this study, we show that Hox alpha and 13-HOTrE exhibit a profound inhibition of MMP-1, -3 and -9 expression by human articular chondrocytes cultured *in vitro*.

Materials and methods

Material

Hox alpha and 13-HOTrE

The dosage form of Hox alpha (Strathmann AG, Hamburg, FRG) contains a stinging nettle leaf extract pelletized with adjuvants. Therefore, an isopropanolic formulation of the "acting agent" (extract) was used for *in vitro* investigations. 13-HOTrE was purchased from Caymann Chemical Inc. (USA).

The monoclonal anti-MMP-1, -MMP-3 and -MMP-9 antibodies (IgG) recognizing both proenzyme and activated enzyme were obtained from R&D Systems (Abingdon, UK). Based on western blot results, these antibodies showed no cross-reactivity with other MMPs. The secondary goat-anti-mouse immunoglobulin antibody conjugated with FITC (GAM-FITC) was purchased from Dianova (Hamburg, FRG). The secondary antibody conjugated to alkaline phosphatase was obtained from Chemicon International, Inc., (Temecula, USA).

Ham's F-12 and DMEM (50/50) were used as a culture medium containing 10% foetal calf serum (FCS), 1% amino acids, 1% L-glutamine, 50 units/ml streptomycin, 50 units/ml penicillin, and 2.5 µg/ml amphotericin B (Biochrom, Berlin, FRG) and 25 µg/ml ascorbic acid (Sigma, München, FRG). Collagen type-II, trypsin (EG 3.4.21.4.) and collagenase (from *Clostridium histolyticum*, 0.15 units/mg) were purchased from Sigma (München, FRG). Interleukin-1ß was obtained from Strathmann Biotec AG (Hannover, FRG).

Methods

Chondrocyte culture

Primary cultures of chondrocytes were prepared from human cartilage as described previously in detail (Shakibaei et al., 1999, 2001). Briefly, full-thickness human articular cartilage specimens from central areas of femoral heads of healthy individuals (obtained during joint replacement surgery for femoral neck fractures) were collected in Ham's F-12 medium. Cartilage slices were rinsed with Hank's solution and digested with 1% pronase (from *Streptomyces griseus*, 7000 units/g) in Hank's solution containing 5% FCS for 2 h at 37 °C and then with 0.2% collagenase in the same solution for 4 h at 37 °C. After rinsing in growth medium, a single cell suspension was obtained by repeated pipetting and separation from undissolved tissue fragments using a nylon mesh with a pore diameter of 80 µm. Cells were sedimented by centrifugation at x6000 g, rinsed twice in growth medium: Ham's F-12/DMEM: 50/50, 10% FCS, 1% amino acids, 1% L-glutamine, 50 units/ml streptomycin, 50 units/ml penicillin, and 2.5 µg/ml amphotericin B, 25 µg/ml ascorbic acid, and resuspended in growth medium at 1.5×10^6 cells/ml. Cultures were kept in an incubator (37 °C) at 95% air and 5% CO_2. Cells were cultured on glass cover slips for immunofluorescence investigations and in petri dishes as monolayer culture for western blot analysis.

Immunofluorescence microscopy

Glass cover slips coated with collagen type-II (500 µg/ml in 0.02N acetic acid at 4 °C overnight), were washed three times with PBS, and then incubated with serum-free medium at 37 °C for 1 h prior to use. Chondrocytes were washed three times with serum-free

medium. After counting, the cells were diluted to 1.5×10^6 cells/ml in serum-free medium, cultured on prepared cover slips for 30 min and then either stimulated with IL-1ß (100 ng/ml for 2 h) or left untreated (negative control) for the indicated times. The stimulated and unstimulated chondrocytes cultured on collagen type-II were then exposed to Hox alpha/13-HOTrE (10 μg/ml) for 24 h. Ethanolic stock solutions of 13-HOTrE and isopropanolic formulation of stinging nettle leaf extract were added directly into the tissue culture media and were solubilized by sonication. The residual content of solvent was lower than 0.2%. This level of solvent was ineffective in affecting the levels of the three investigated MMPs. For immunofluorescence microscopy, cells were fixed in 1% paraformaldehyde and rinsed in PBS for 3x5 min. Cells were incubated overnight with the primary antibodies (anti-MMP-1, -3 and -9 antibodies, diluted 1:50 in PBS) in a moist chamber at 4 °C and were incubated with the secondary antibody GAM-FITC (diluted 1:50) for 1 h at RT. Between each step cells were washed twice with PBS/BSA. Finally, they were washed with distilled water (30 min), air-dried, covered with Fluoromount mountant and evaluated under a light microscope (Axiovert 100, Zeiss, Jena).

Western blot analysis for MMPs

A detailed description of the culture technique used for the following experiments has been published previously (Shakibaei et al., 1999, 2001; Schulze-Tanzil et al., 2001). Briefly, tissue culture dishes were coated with collagen type-II (500 μg/ml in 0.02N acetic acid), washed three times with PBS, and then incubated with serum-free medium at 37 °C for 1 h prior to use. Human chondrocytes were washed three times with serum-free medium. After counting, the cells were diluted to 1.5×10^6/ml in serum-free medium, plated on dishes coated with collagen type-II for 30 min, then either stimulated with IL-1ß (100 ng/ml) or left untreated for 2 h. The stimulated and unstimulated (controls) chondrocytes cultured on collagen type-II were then exposed to Hox alpha or 13-HOTrE (10 μg/ml) for 24 h as described before. After rinsing with PBS, cells were treated with lysis buffer (50 mM Tris-HCl pH 7.2, 150 mM NaCl, 1% Triton X-100, 1 mM sodium orthovanadate, 50 mM sodium pyrophosphate, 100 mM sodium fluoride, 0.01% aprotinin, 4 μg/ml pepstatin A, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride [PMSF]) on ice for 30 min. Insoluble material was removed by centrifugation at 13,000 rpm for 30 min. Lysates were stored at -70 °C until use. For immunoblotting, equal amounts of total protein were separated on 7.5% or 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS)-polyacrylamide gels under reducing conditions. Proteins were transferred for 80 min at 120 V onto nitrocellulose (Schleicher & Schüll, Dassel, FRG) using a transblot electrophoresis apparatus (Mini Trans Blot™, BioRad

Laboratories, Richmond, USA). Membranes were blocked with 5% (w/v) skimmed milk powder in PBS/0.1% Tween 20 overnight at 4 °C and incubated with primary antibodies diluted in blocking buffer for 1 h at RT. After 5 washes in blocking buffer, membranes were incubated with alkaline phosphatase-conjugated secondary antibody diluted in blocking buffer for 30 min at RT. Membranes were finally washed 5 times in blocking buffer, twice in 0.1 M Tris, pH 9.5, containing 0.05 M MgCl_2 and 0.1 M NaCl. Specific binding was detected using nitro blue tetrazolium and 5-bromo-4-chloro-3-indoyl-phosphate (*p*-toluidine salt; Pierce, Rockford, IL, USA) as substrates and quantified by densitometry (IP Lab Gel software, Signal Analysis Corporation, VA, USA). Protein determination was done with the bicinchoninic acid system (Pierce, Rockford, IL, USA) using BSA (bovine serum albumine) as a standard.

Results

Chondrocyte culture

Cells grown on collagen type-II always exhibited a round to oval shape and numerous small ridge-like or cusp-like surface processes from the beginning of cultivation onwards. Development of chondrocytes in culture was observed daily during the culture period, as described by Shakibaei (1995) and Shakibaei et al. (1997, 1999).

Effects of IL-1ß on MMP expression on human chondrocytes

MMP expression was found diffusely distributed on the round chondrocytes cultured on collagen type-II (Fig. 1A-C). To see the effect of IL-1ß on the expression of MMPs on chondrocytes, cells were cultured on collagen type-II-coated petri dishes, stimulated with IL-1ß for 2 h, and then immunolabelled against MMP-1, -3, -9 (Fig. 2A-C). As shown in Fig. 2A-C, the chondrocytes grown on collagen type-II and treated with IL-1ß exhibited a higher expression of MMPs than those cultivated on collagen type-II which were not treated with IL-1ß (Fig. 1A-C). These results indicate a specific positive upregulatory effect of IL-1ß on the expression of MMPs on human chondrocytes. These findings confirm the previous results (Horton et al., 1998; Hui et al., 1998; Robbins et al., 2000).

Effects of Hox alpha/13-HOTrE on MMP expression on human chondrocytes

To investigate the effect of Hox alpha/13-HOTrE on MMP expression of IL-1ß-treated and untreated chondrocytes cultured on collagen type-II, Hox alpha/13-HOTrE was added to the medium (10 μg/ml). After 24 h of cultures, the cells were immunolabelled using MMP-1, -3 and -9 antibodies. As figures 1D-F and

2D-F show the expression of all 3 MMPs on the surface of chondrocytes grown on collagen type-II, non-stimulated with IL-1ß (control) (1D-F) and stimulated with IL-1ß (2D-F) and then treated with Hox alpha (13-HOTrE, data not shown) was significantly lower than that of those cultivated on collagen type-II without Hox alpha/13-HOTrE exposure. These results indicate a significant inhibition of the expression of these MMPs by Hox alpha/13-HOTrE in human chondrocytes cultured *in vitro*.

Western blot analysis

Parallel to immunofluorescence microscopy, the immunoblotting method for the detection of MMP expression by IL-1ß-stimulated or non-stimulated (control) human chondrocytes cultured on collagen type-II, exposed to Hox alpha/13-HOTrE (10 µg/ml) was employed. After 24 h of treatment, the cells were lysed and analysed by western blot. In the presence of Hox alpha/13-HOTrE the basal expression of MMP-1, -3 and -9 was slightly inhibited (Fig. 3A-C, lanes 2 and 3). Stimulation of the cultures with 100 ng/ml IL-1ß for 2h resulted in a markedly increased expression of all three investigated MMPs (Fig. 3A-C, lane 4). Coincubation with Hox alpha/13-HOTrE also strongly decreased the IL-1ß-stimulated expression of MMP-1, MMP-3 and MMP-9 (Fig. 3A-C, lanes 5 and 6), compared to untreated controls (lane 4).

Densitometric evaluations

Next, densitometric evaluation of a representative experiment performed in triplicate from western blot analysis of the detection of MMP expression by non-stimulated (control) human chondrocytes cultured on collagen type-II, exposed to 13-HOTrE/Hox alpha showed that the relative MMP-1 expression decreased by 46% (p=0.034) / 49% (p=0.017); MMP-3 by 94% (p=0.00016)/99% (p=0.017); MMP-9 by 96% (p=0.00026)/100% (p=0.15) compared to chondrocytes cultured on collagen type-II and in absence of 13-
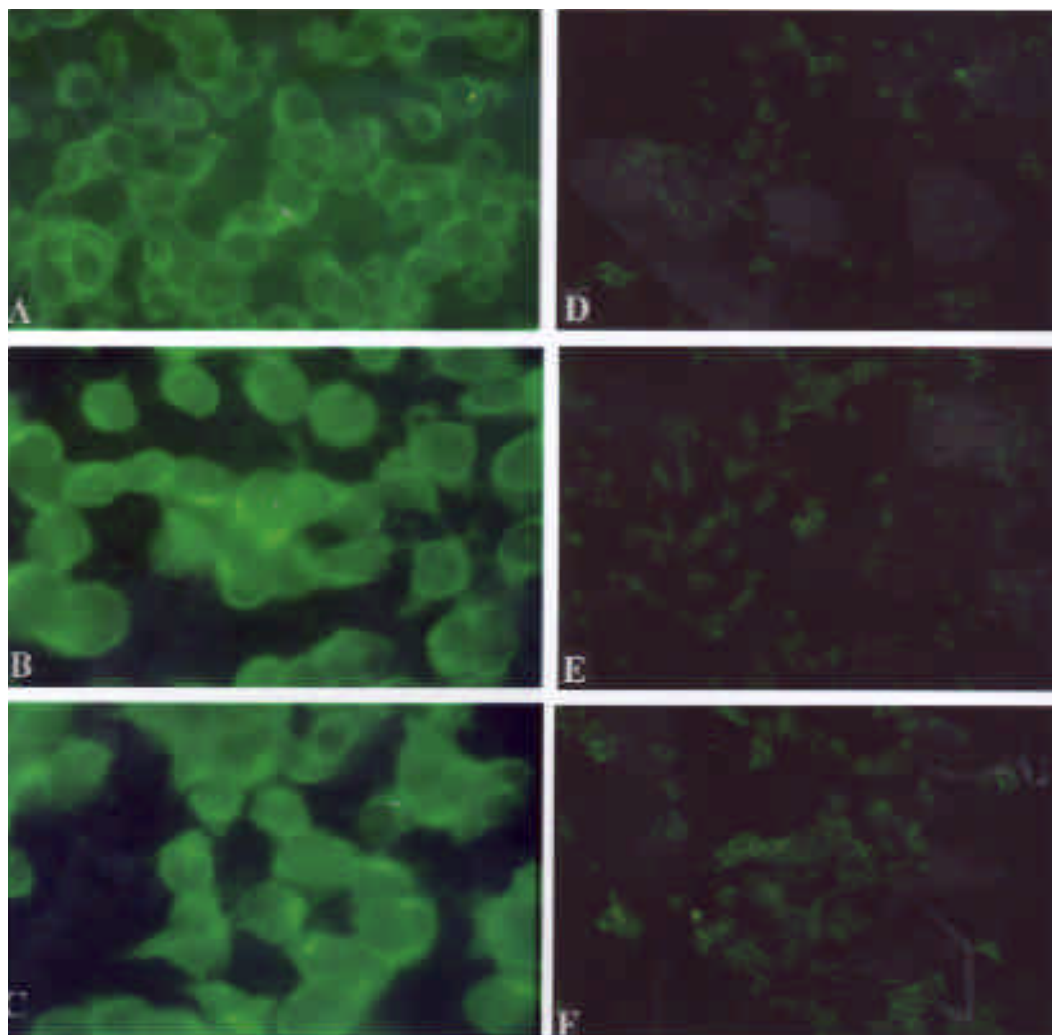

Fig. 1. Effects of Hox alpha on MMP expression of chondrocytes. Immunofluorescence labelling of MMPs on human chondrocytes cultured in monolayer culture on collagen type-II and treated with Hox alpha **(D, E, F)** or left untreated **(A, B, C)**. Chondrocytes are stained with monoclonal antibodies against MMP-1 **(A, D)**, MMP-3 **(B, E)** and MMP-9 **(C, F)** and with FITC. Immunolabellings of MMP-1, MMP-3 and MMP-9 are restricted to the cell surface and exhibit almost the same distribution but decrease clearly after Hox alpha treatment **(D-F)**. x 160

HOTrE/Hox alpha. Densitometric evaluation of the results from western blot analysis of the detection of MMP expression by IL-1ß-stimulated human chondrocytes cultured on collagen type-II, exposed to 13-HOTrE/Hox alpha showed that the relative MMP-1 expression decreased by 63% (p=0.0057)/60% (p=0.054); MMP-3 by 91% (p=0.0016)/88% (p=0.029); MMP-9 by 96% (p=0.0014)/88% (p=0.0078) compared to chondrocytes cultured on collagen type-II and stimulated with IL-1ß in the absence of 13-HOTrE/Hox alpha.

Discussion

The goal of the study presented here was to show the effects of a new antirheumatic drug; Hox alpha (lipophilic stinging nettle leaf extract) and an isolated cytokine-suppressive component 13-HOTrE, on *in vitro* MMP expression in human chondrocytes. Under the experimental conditions, we found: (1) expression of MMPs (MMP-1, -3, -9) on the chondrocyte surface; and

(2) Hox alpha or 13-HOTrE significantly decreased the MMP expression on with IL-1ß-stimulated and non-stimulated human chondrocytes cultured on collagen type-II *in vitro*.

It is well known that the interaction of chondrocytes and their matrix is important for the proliferation, differentiation and survival of cells (Kosher et al., 1973; Kosher and Church, 1975; Hewitt et al., 1982; Sommarin et al., 1989; Shakibaei et al., 1997, 1999). These processes require a permanent remodelling (turnover) of extracellular matrix proteins executed by MMPs (Schulze-Tanzil et al., 2001), a group of zinc-dependent endopeptidases that cleave ECM molecules. Imbalance between MMPs and their natural inhibitors (TIMPs) results in pathological conditions in the cartilage (Dean et al., 1989; Martel-Pelletier et al., 1994) and indeed MMPs play an important role in cartilage destruction in arthritis. Several studies have reported that high levels of different MMPs (MMP-1, MMP-3 and MMP-9) were found in the synovium and in the serum of osteoarthritic and rheumatoid arthritis patients
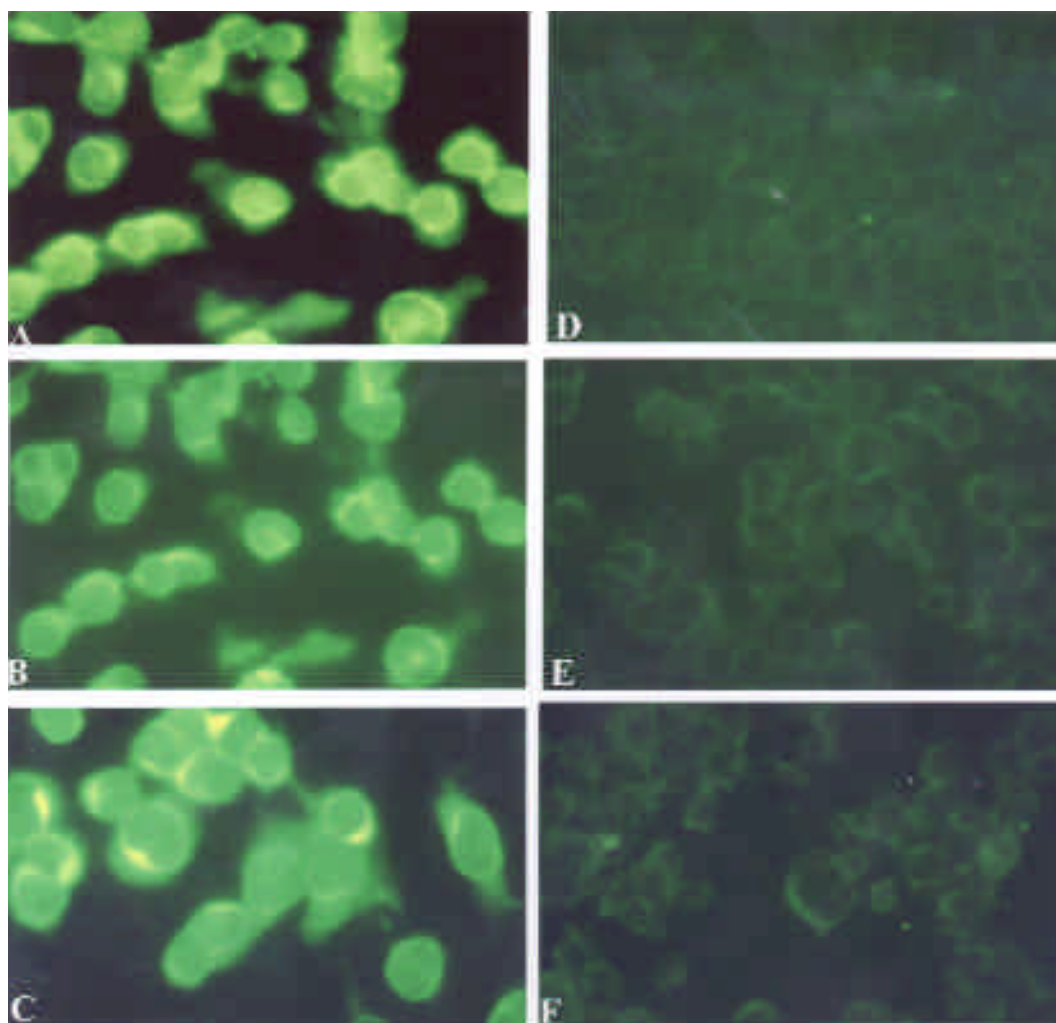

Fig. 2. Effects of Hox alpha on MMP expression of with IL-1ß stimulated chondrocytes. Human chondrocytes are plated on dishes coated with collagen type-II and then stimulated with IL-1ß. Chondrocytes are treated with Hox alpha **(D, E, F)** or left untreated **(A, B, C)**. They are stained with monoclonal antibodies against MMP-1 **(A, D)**, MMP-3 **(B, E)** and MMP-9 **(C, F)** and with FITC. The expression of the MMP-1 **(A)**, MMP-3 **(B)** and MMP-9 **(C)** increases in the presence of IL-1ß but rapidly decreases after treatment with Hox alpha **(D-F)**. Immunolabelling of MMP-1, MMP-3 and MMP-9 is restricted to the cell surface and exhibits almost the same distribution. x 160

(Kolkenbrock et al., 1993; Manicourt et al., 1995; Freemont et al., 1997; Keyszer et al., 1999). Collagen degradation plays a pivotal role in osteoarthritic cartilage and is mediated by collagenases such as MMP-1, MMP-8 and MMP-13 (Neuhold et al., 2001). Especially, MMP-13 is suggested to be primarily involved in collagen cleavage, which is an indication of irreversible osteoarthritic changes. In fact, specific cleavage of aggrecan occurs during the early stages of osteoarthritic development (Arner et al., 1998). There are two but independent catabolic pathways of cleavage of aggrecan: by MMPs or by aggrecanases (Tortorella et al., 2000). Aggrecanases do not fullfill all characteristics of the MMP family and therefore are assigned to ADAM-TS (a disintegrin and metalloprotease with thrombospondin motif) family of metalloproteinases (Arner et al., 1999). Bluteau et al. (2001) suggested a differential regulation of mRNA levels of MMPs (MMP-1, -3, -13) and aggrecanases (aggrecanase-1 and -2) in response to IL-1ß. Despite the importance of aggrecanases in arthritis we restricted this study to MMPs.

The results of immunofluorescence experiments and quantitative densitometry from western blots implicate cytokine IL-1ß regulation in MMP expression in cartilage, which confirms the previous studies (Horton et al., 1998; Robbins et al., 2000). Many proinflammatory cytokines such as TNF- and IL-1ß are overexpressed in the synovium of rheumatoid arthritis and osteoarthritis patients (Feldmann et al., 1996a; Martel-Pelletier, 1998),

and these cytokines play a very important role in the pathogenesis of rheumatoid arthritis (Brennan et al., 1992).

Beside standard therapy blockade of cytokines, e.g. by cytokine antibodies or soluble cytokine receptors, this is a relatively new clinical therapeutical approach in treatment of inflammatory and degenerative cartilage diseases but this therapy is expensive and suspected to exhibit serious adverse effects because long term safety data are lacking (Kremer, 2001; Seymour et al., 2001). On the other hand, different classes of MMP inhibitors are under investigation for rheumatoid arthritis therapy, e.g. zinc chelating/binding agents (de Bri et al., 1998; Skotnicki et al., 1999; Hamada et al., 2000). However results from clinical trials in rheumatoid arthritis have been equivocal (Elliot and Cawston, 2001).

Recently, the oxylipin 13-Hydroxyoctadecatrienic acid (13-HOTrE) has been isolated from stinging nettle leaf extracts as a cytokine-suppressive agent by using different *in vitro* models (Klingelhoefer et al., unpublished data). Hox alpha, a newly-developed lipophilic stinging nettle leaf extract, has a higher content of 13-HOTrE which correlates to a stronger cytokine-inhibiting efficacy *in vitro* (Klingelhoefer et al., unpublished data).

Here, we have demonstrated by immunofluorescence microscopy and western blot analysis *in vitro* that the newly-developed extract, Hox alpha, potently decreases basal and IL-1ß-stimulated expression of MMPs (MMP-
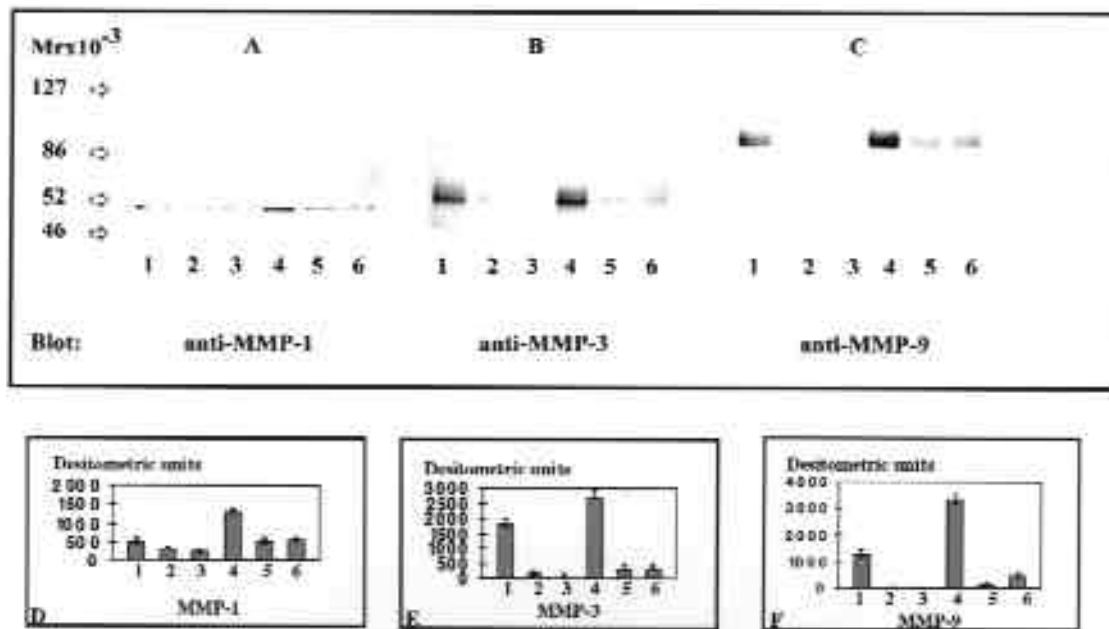

Fig. 3. Western blot analysis. Human chondrocytes are plated on dishes coated with collagen type-II, either stimulated with IL-1ß (100 ng/ml) (lane 4, 5 and 6) or left unstimulated (lane 1, 2 and 3) and then treated with 13-HOTrE (10 ng/ml) (lane 2 and 5) or Hox alpha (10 ng/ml) (lane 3 and 6) or left untreated (lane 1 and 4) for 24 hours. Equal amounts (50 µg of protein per lane) of total proteins are separated by SDS-PAGE and analysed by immunoblotting with anti-MMP-1 **(A)**, -MMP-3 **(B)**, -MMP-9 **(C)** antibodies. Lane 1: Chondrocytes cultivated on collagen type-II, without IL-1ß. Lane 2: Chondrocytes cultivated on collagen type-II, without IL-1ß, exposed to 13-HOTrE. Lane 3: Chondrocytes cultivated on collagen type-II, without IL-1ß, exposed to Hox alpha. Lane 4: Chondrocytes cultivated on collagen type-II, stimulated with IL-1ß. Lane 5: Chondrocytes cultivated on collagen type-II, stimulated with IL-1ß, exposed to 13-HOTrE. Lane 6: Chondrocytes cultivated on collagen type-II, stimulated with IL-1ß, exposed to Hox alpha. **D-F.** Densitometric evaluations. Quantitative densitometry demonstrates prolonged peak expression of MMP-1 (**D**, lane 4), -3 (**E**, lane 4) and -9 (**F**, lane 4) occurring with IL-1ß stimulation. In the presence of Hox alpha / 13-HOTrE the basal expression of MMP-1, -3 and -9 is slightly inhibited in stimulated and unstimulated cultures (**D-F**, lanes 2 and 3). Values are means ± SD of a representative experiment performed in triplicate. Data shown are representative of three independent experiments.

1, -3 and -9). Furthermore, the isolated cytokine-suppressive component 13-HOTrE was also effective at a similar concentration. It has been reported that an extract from stinging nettle leaves (IDS23) suppresses cytokine production of T-cells mainly through inhibition of IL-2 and IFN-gamma gene expression at the transcriptional level (Klingelhoefer et al., 1999). In fact, Riehemann et al. (1999) have shown that treatment of different cells with IDS23 inhibits nuclear factor-κB (NF-κB) activation in response to several stimuli. NF-κB is activated in several chronic inflammatory diseases, such as rheumatoid arthritis (Handel et al., 1995; Marok et al., 1996; Miyazawa et al., 1998). Proinflammatory cytokines, such as TNF-α and IL-1ß, induce translocation of NF-κB to the nucleus in chondrocytes (Ding et al., 1998). Other transcription factors which have been implicated in the pathogenesis of rheumatoid arthritis are AP-1 and Ets-1 (Ashara et al., 1997; Shiozawa et al., 1997; Hui et al., 1998; Redlich et al., 2001). Besides proinflammatory cytokines, target genes of NF-κB and AP-1 include the matrix proteinase family of proteins (Hui et al., 1998; Vincenti et al., 1998; Bondeson et al., 1999; Barchowsky et al., 2000; Eberhardt et al., 2000; Mengshol et al., 2000). NF-κB might also be responsible for downregulation of the transcription factor sox9, which is involved in the regulation of genes for cartilage-specific ECM proteins (Murakami et al., 2000).

These results support the hypothesis that the diverse effects of stinging nettle leaf extracts are mediated by the same targets in different cell types; namely proinflammatory transcription factors such as NF-κB and AP-1. Besides, other unknown contents of Hox alpha extract with additive and synergistic effects, 13-HOTrE may be a component of major importance for the extract activity. Therefore, Hox alpha and 13-HOTrE may represent a promising remedy for arthritis therapy since Hox alpha seems to have the advantage that it simultaneously impairs cytokine and matrix metalloproteinase gene transcription via NF-κB in chondrocytes and therefore interferes with early pathomechanisms of rheumatoid arthritis. Further investigations are needed to elucidate the cellular targets of these agents.

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