Osteoarthritis and Cartilage (2001) **9,** 257–263
© 2001 OsteoArthritis Research Society International
doi:10.1053/joca.2000.0383, available online at http://www.idealibrary.com on IDEAL®
1063–4584/01/030257+07 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Rhein, an active metabolite of diacerein, down-regulates the production of pro-matrix metalloproteinases-1, -3, -9 and -13 and up-regulates the production of tissue inhibitor of metalloproteinase-1 in cultured rabbit articular chondrocytes

T. Tamura*, N. Kosaka*, J. Ishiwa†, T. Sato†, H. Nagase‡ and A. Ito†
**Drug Development Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd, Shimotogari, Nagaizumi, Sunto, Shizuoka, Japan; †Department of Biochemistry and Molecular Biology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Horinouchi, Hachioji, Tokyo, Japan; and ‡The Kennedy Institute of Rheumatology, Imperial College School of Medicine, Hammersmith, London, U.K.*

Summary

Objective: Diacerein has proved to be effective in the treatment of osteoarthritis (OA). However, the precise action mechanism of diacerein on OA is not yet fully understood. Therefore, we investigated the effects of rhein, an active metabolite of diacerein, on the production of promatrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase-1 (TIMP-1) in rabbit articular chondrocytes.

Design: Confluent rabbit chondrocytes were treated for 24 or 48 h with rhein, naproxen or dexamethasone in the presence of recombinant human IL-1α (rhIL-1α). ProMMP-9/progelatinase B in the culture medium was monitored by gelatin zymography, and proMMP-1/procollagenase 1, proMMP-3/prostromelysin 1, proMMP-13/procollagenase 3 and TIMP-1 were analysed by Western blot analysis. The steady-state levels of proMMP mRNAs were examined by Northern blot analysis. Total MMPs activity was also determined using FITC-labeled casein.

Results: Rhein suppressed the rhIL-1α-induced production of proMMPs-1, -3, -9 and -13 in a dose-dependent manner (0.1–30 μM). The suppressed production of proMMPs-1 and -3 was accompanied by a decrease in the steady-state levels of their mRNAs. Interestingly, rhein increased the production of TIMP-1. These observations were further supported by the fact that rhein decreased the apparent total activity of MMPs in the culture medium.

Conclusion: We have demonstrated that rhein, an active metabolite of diacerein down-regulates the gene-expression and production of proMMPs and up-regulates the TIMP-1 production. The therapeutic effects of diacerein on OA may be due, at least in part, to the chondroprotective effect of rhein, its active metabolite. © 2001 OsteoArthritis Research Society International

Key words: Diacerein, Rhein, Chondrocytes, Matrix metalloproteinases.

Introduction

Osteoarthritis (OA) is a degenerative process of the joints characterized by progressive destruction and erosion of cartilage associated with an osteophytic response. This destruction results from a homeostatic imbalance between the matrix synthesis and degradation in the cartilage. Numerous studies have demonstrated that matrix metalloproteinases (MMPs) are most frequently implicated in the destruction of articular cartilage in arthritic diseases[1,2]. MMP-1/collagenase 1, MMP-3/stromelysin 1, MMP-9/gelatinase B and MMP-13/collagenase 3 have been detected in the synovium, synovial fluid, and cartilage samples with rheumatoid arthritis (RA) and with OA, and closely participate in the pathologic loss of articular cartilage[3–8]. Furthermore, pro-inflammatory cytokines, which are produced by monocytes/macrophages, synovial cells or chondrocytes in the arthritic area of the joint,[9] induce and/or augment the production of proMMPs[10–12]. The activities of MMPs are controlled at several steps including proenzyme activation and inhibition by tissue inhibitors of metalloproteinases (TIMPs)[13]. Therefore, the resultant excess molar of MMPs over TIMPs is important during the cartilage breakdown in arthritic diseases[14].

Clinical studies have suggested that diacerein (4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid) exerts a beneficial effect on the symptoms of OA[15–17]. The modes of action of diacerein toward OA have been examined by many investigators; e.g. diacerein shows anti-osteoarthritic and chondroprotective effects in animal OA models[18,19], and augments hyaluronan synthesis in synovial cells[20]. Diacerein also prevents cartilage breakdown by a reduction of the proinflammatory cytokines[21]. Diacerein is known to be completely metabolized by animals and humans into rhein (4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid) which is an active metabolite of diacerein[22], and rhein reduces the production of superoxide anion in human neutrophils[23]. However, the exact mechanisms of the therapeutic action of diacerein on OA are not clear.

Received 10 February 2000; revision requested 16 June 2000; revision received 9 August 2000; accepted 20 September 2000.
Address correspondence to: Tadafumi Tamura, Drug Development Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd, 1188 Shimotogari, Nagaizumi, Sunto, Shizuoka 411-8731, Japan. Tel: 81-559-89-2011; Fax: 81-559-86-7430; E-mail: tadafumi.tamura@kyowa.co.jp

Therefore, we investigated the effects of rhein, an active metabolite of diacerein, on the production of pro-MMPs including proMMPs-1, -3, -9 and -13 and TIMP-1 in rabbit articular chondrocytes to obtain further insight into the clinical application of this agent.

Material and methods

CULTURE OF RABBIT ARTICULAR CHONDROCYTES

Articular chondrocytes were isolated by the method of Green[24] with slight modifications from the shoulder and knee joints of 3-week-old female Japanese White rabbits (Tokyo Experimental Animal, Tokyo, Japan). Cartilage was digested with 0.125% trypsin (Difco Laboratories, Detroit, MI) for 1 h and with 0.1% collagenase (Worthington Biochemical, Freehold, NJ) for 2 h. Chondrocytes were then suspended in Dulbecco's modified Eagle's medium (DMEM; Life Technologies Inc., Rockville, MD) with 10% fetal bovine serum (FBS; BioWhittaker, Walkersville, MD) and antibiotics (100 units/mL penicillin, 100 μg/mL streptomycin and 250 ng/mL fungizone®, Life Technologies). The chondrocytes were cultured in 24-multiwell tissue culture plates (FALCON®, Becton Dickinson Labware, Franklin Lakes, NJ) or in 100-mm tissue culture dishes (Iwaki Glass, Chiba, Japan) at a density of 2.5×10^4 cells/cm^2 in 5% CO_2-95% air at 37°C. Primary cultures maintained in a monolayer were used throughout the study. After cells reached confluence, the medium was changed to DMEM with 0.2% lactalbumin hydrolysate (Sigma Chemical, St Louis, MO) and the antibiotics. Confluent chondrocytes were then incubated with rhein sodium salt (Kyowa Hakko Kogyo, Tokyo, Japan), naproxen (Sigma) or dexamethasone (Sigma) in the presence of 1 ng/mL of recombinant human IL-1α (rhIL-1α; Genzyme, Cambridge, MA) for 24 or 48 h. To make stock solutions, rhein sodium salt was dissolved in distilled water at 3×10^{-2} M, and naproxen and dexamethasone were dissolved in dimethyl sulfoxide at 1×10^{-1} M, and they were added to the cell culture at appropriate concentrations. The final concentrations of distilled water and dimethyl sulfoxide were 0.1% and 0.01%, respectively. The concentrations of rhein used were chosen to reflect levels that are below, at, or above the established therapeutic antiinflammatory serum values of rhein after dosing with diacerein as described in clinical practice[22,25], since diacerein is known to be completely metabolized into rhein[22]. The harvested culture media were stored at −40°C until use.

GELATIN ZYMOGRAPHY

ProMMP-9 in the conditioned medium was detected by gelatin zymography as previously described[26]. The samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% acrylamide gel containing 0.6 mg/mL of gelatin (Difco Laboratories) under non-reducing conditions at 4°C. After electrophoresis, SDS in the gel was removed by rinsing with 50 mM Tris-HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.02% NaN_3, and 2.5% Triton-X 100 (pH 7.5). The gel was then incubated for 2 h at 37°C in the same buffer without Triton-X 100. After incubation, the gel was stained with 0.1% Coomassie brilliant blue R250 (Bio-Rad Laboratories, Hercules, CA) in 50% methanol/20% acetic acid, and destained with 1% formic acid/30% methanol. The gelatinolytic activity was analysed using National Institute of Health Image software (NIH, Bethesda, MD).

WESTERN BLOT ANALYSIS

The levels of MMPs-1, -3, -13 and TIMP-1 in the conditioned medium were analysed by Western blot analysis. Sheep anti-(rabbit proMMP-1 or proMMP-3) antibody, or sheep anti-(human TIMP-1) antibody were prepared as previously described[27,28] and rabbit anti-(human proMMP-13) antibody was kindly provided by Dr P. Mitchell[29]. The collected conditioned medium was subjected to SDS-PAGE with 12.5% (proMMP-1 and TIMP-1) or 10% (proMMP-3 and proMMP-13) acrylamide gels under reducing conditions. After electrophoresis, proteins in the gel were electrotransferred onto a nitrocellulose membrane (Protran®; Schleicher and Schuell, Dassel, Germany). The membrane was then blocked, washed, and incubated overnight with sheep anti-(rabbit proMMP-1 or proMMP-3) antibody, rabbit anti-(human proMMP-13) antibody or sheep anti-(human TIMP-1) antibody which was then complexed with alkaline phosphate conjugated donkey anti-sheep IgG (Sigma) for 2 h or peroxidase conjugated anti-rabbit or -sheep IgG (Sigma) for 1 h. The immunoreactive proMMP-1 and proMMP-3 were indirectly visualized using 5-bromo-4-chloro-3-indolyl phosphate (Sigma) and nitro blue tetrazolium (Sigma) (Conventional Western blotting) as previously described[30], and proMMP-13 and TIMP-1 were monitored by enhanced chemiluminescence (ECL)-Western blotting detection reagents (Amersham Pharmacia Biotech, Tokyo, Japan) as previously described[31].

ASSAY FOR CASEINOLYTIC ACTIVITY OF MMPS

The apparent MMPs activity in the conditioned medium was determined using FITC-labeled casein as a substrate, according to the method of Twining[32], with slight modifications for microassay. Before the assay, proMMPs in the conditioned medium were activated by TPCK treated-trypsin (0.1 mg/mL, Worthington Biochemical) followed by the addition of four molar excess of soybean trypsin inhibitor (Life Technologies). The culture medium (80 μL) and 0.5% FITC-labeled casein were incubated in a total 130 μL of 0.5 M Tris-HCl, pH 7.2, for 42 h at 37°C. After the incubation the reaction was terminated by adding trichloroacetic acid at final concentration of 6.8%. The mixtures were centrifuged at 4°C and 2000 rpm for 15 min. A portion (50 μL) of the supernatant was mixed with 200 μL of the assay buffer (0.5 M Tris-HCl, pH 8.5), and fluorescence was subsequently measured at 485 nm excitation and 530 nm emission wavelengths using a Cyto Fluor™ fluorescence multiwell plate reader (PerSeptive Biosystems, Tokyo, Japan). The enzyme activity that digests 1 μg of casein for 1 h at 37°C was expressed as one unit.

NORTHERN BLOT ANALYSIS

The steady-state levels of proMMP mRNA were determined by Northern blot analysis as previously described[33]. Complementary DNA of human proMMP-1 (1.6 kb) and human proMMP-3 (1.4 kb) were prepared as previously described[34]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was purchased from Clontech Laboratories (Palo Alto, CA). Human proMMP-1, human proMMP-3 or GAPDH cDNA was labeled with [α-^{32}P]dCTP (DuPont

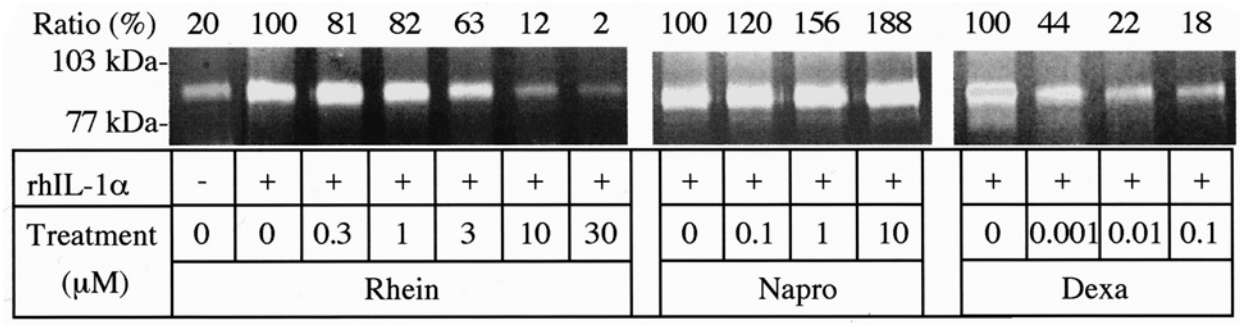

Fig. 1. Effects of rhein, naproxen and dexamethasone on the production of proMMP-9. Confluent chondrocytes in 24-well plates were treated with rhIL-1α (1 ng/mL) in the presence and absence of the indicated drug for 48 h, and then the conditioned culture medium was subjected to gelatin zymography as described in the text. Three independent experiments using cells of different origins were highly reproducible, and typical data are shown. The relative amounts of proMMP-9 were quantified by densitometric scanning and shown at the top of the panels by taking the rhIL-1α-treated cells as 100. Napro; naproxen and Dexa; dexamethasone.

NEN, Boston, MA) using a random primed DNA labeling kit (Boehringer Mannheim, Indianapolis, IN). Total cytoplasmic RNA was extracted using ISOGEN (Nippon Gene Co., Toyama, Japan) according to the manufacturer's instructions and stored at −80°C. The isolated RNA (20 μg) was denatured and electrophoresed on 1% formaldehyde-denatured agarose gel and then transferred onto a nylon membrane (GeneScreen; NEN™ Life Science Products, Boston, MA). The membrane was hybridized with each ^{32}P-labeled cDNA for proMMPs-1, -3 or GAPDH probe at 42°C in the hybridization solution containing 50% formamide, 5×saline sodium citrate (SSC), 100 μg/ml denatured salmon sperm DNA, 1×Denhard's solution, and 0.1% SDS. After hybridization, the membrane was sequentially washed in 2×SSC/0.1% SDS at room temperature and subjected to autoradiography using Konica medical X-ray film (Konica Co., Tokyo, Japan) at −80°C. The resulting autoradiogram was analysed with NIH Image software and normalized to the relative intensities of the corresponding GAPDH band.

STATISTICAL ANALYSIS

The values of MMPs activity determined in the conditioned medium were expressed as the mean with standard deviation for each group. The comparison was analysed by F test followed by Aspin–Welch test. Multiple comparisons between treatment groups were assessed by a one-way analysis of variance followed by Dunnett's test. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

EFFECTS OF RHEIN ON THE RHIL-1α-INDUCED PROMMPS-9, -1, -3, -13 AND TIMP-1 PRODUCTION

Gelatin zymography indicated that rabbit articular chondrocytes spontaneously produced small amounts of proMMP-9. When confluent chondrocytes were treated with rhIL-1α (1 ng/mL) for 48 h, the level of proMMP-9 in the conditioned medium increased by about five-fold as compared with that of the untreated control cells (Fig. 1). Rhein suppressed the rhIL-1α-induced proMMP-9 production in a dose-dependent manner (0.1–30 μM) with a maximal inhibition of 98% at 30 μM. Dexamethasone (1–100 nM) effectively suppressed the production of proMMP-9, whereas naproxen (0.1–10 μM) enhanced it.

We further examined the effects of rhein on the rhIL-1α-induced production of proMMPs-1, -3 and -13 by Western blot analysis. As shown in Fig. 2, rhIL-1α (1 ng/mL) markedly increased the secreted protein level of proMMPs-1, -3 and -13. Rhein inhibited the production of proMMPs-1, -3 and -13 in a dose-dependent manner (3–30 μM) with maximum 88, 82 and 100% inhibitions at 30 μM, respectively. Dexamethasone also suppressed the production of these proMMPs in a dose-dependent manner (1–100 nM). By contrast, naproxen (1–10 μM) slightly enhanced the production of proMMPs-1 and -3, but not proMMP-13.

Next, we investigated the effect of rhein on the TIMP-1 production by Western blot analysis. When confluent chondrocytes were treated with rhIL-1α (1 ng/mL) for 24 h, the production of TIMP-1 increased by about three-fold as compared with that of the untreated control cells (Fig. 3). Neither naproxen nor dexamethasone modulated the TIMP-1 production. By contrast, rhein further augmented the rhIL-1α-induced TIMP-1 production in a dose-dependent manner (10–30 μM) with a maximum augmentation of 222% at 30 μM.

EFFECTS OF RHEIN ON THE RHIL-1α-ENHANCED CASEINOLYTIC ACTIVITY OF MMPS

Since the expression of the MMP activities depends on the balance between MMPs and TIMPs, we investigated whether the effects of rhein resulted in a decrease in the apparent MMPs activity. The proMMPs in the conditioned medium were activated by exposure to TPCK-treated trypsin. Recombinant hIL-1α (1 ng/mL) significantly augmented the caseinolytic activity compared with the untreated control ($P<0.0001$ at 24 h and $P=0.0001$ at 48 h) (Table I). Rhein (30 μM) significantly suppressed the rhIL-1α-enhanced activity ($P=0.0058$, 39% inhibition at 24 h and $P=0.0241$, 36% inhibition at 48 h). Dexamethasone (10 nM) also showed significant inhibition ($P=0.0002$, 62% inhibition at 24 h and $P=0.0050$, 32% inhibition at 48 h) whereas naproxen (1 μM) had no effect. In addition, it was investigated whether rhein had a direct inhibitory effect on the caseinolytic activity. The culture medium of rhIL-1α-treated chondrocytes was used as a source of enzyme and was TPCK-treated trypsin to activate proMMPs. Rhein

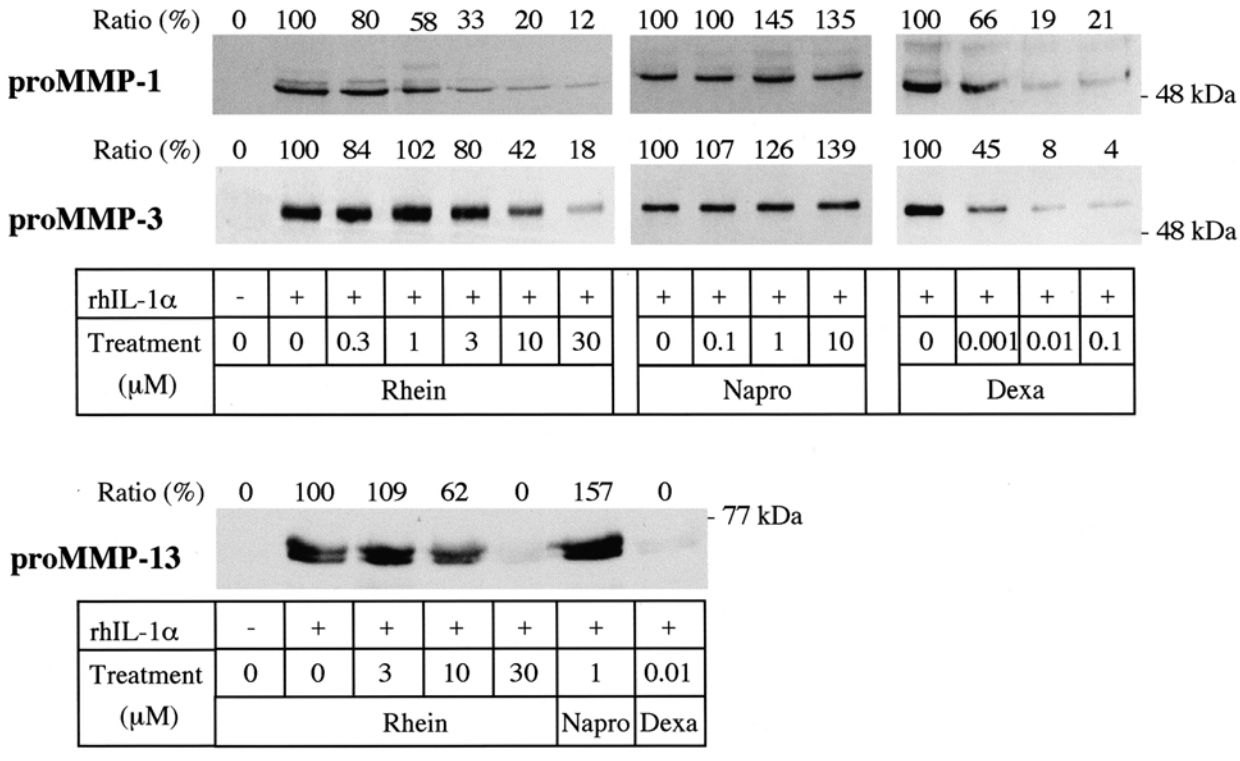

Fig. 2. Effects of rhein, naproxen and dexamethasone on the production of proMMPs-1, -3 and -13. ProMMP-1 and proMMP-3; confluent chondrocytes in 24-well plates were treated with rhIL-1α (1 ng/mL) in the presence and absence of the indicated drug for 48 h. The culture medium was subjected to conventional-Western blot analysis as described in the text. Pro-MMP-13; Confluent chondrocytes in 100-mm dishes were treated with rhIL-1α (1 ng/mL) and drugs for 24 h. The culture medium was subjected to ECL-Western blot analysis as described in the text. Three independent experiments using cells of different origins were highly reproducible, and typical data are shown. The relative amounts of pro-MMPs-1, -3 and -13 were quantified by densitometric scanning and shown at the top of the panels by taking the rhIL-1α-treated cells as 100. Napro; naproxen and Dexa; dexamethasone.

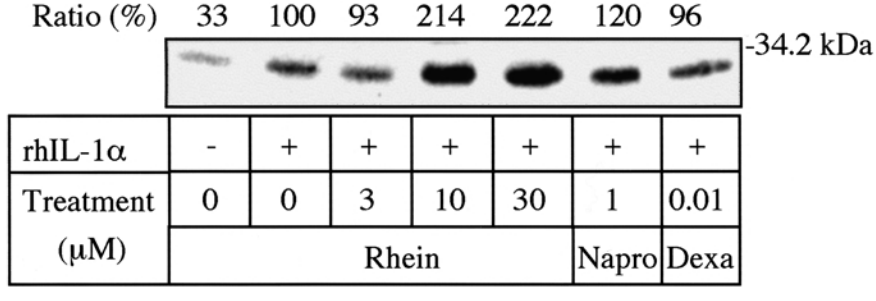

Fig. 3. Effects of rhein, naproxen and dexamethasone on the production of TIMP-1. Confluent chondrocytes in 100-mm dishes were treated with rhIL-1α (1 ng/mL) in the presence and absence of the indicated drug for 24 h. The culture medium was subjected to ECL-Western blot analysis as described in the text. Five independent experiments using cells of different origins were highly reproducible, and typical data are shown. The relative amounts of TIMP-1 were quantified by densitometric scanning and shown at the top of the panels by taking the rhIL-1α-treated cells as 100. Napro; naproxen and Dexa; dexamethasone.

did not directly modulate the caseinolytic activity (data not shown). These results further supported the fact that both rhein and dexamethasone inhibited the production of proMMPs.

RHEIN SUPPRESSES THE RHIL-1α-INDUCED EXPRESSION OF PRO-MMP MRNA

We further investigated whether the suppression by rhein of the production of proMMPs-1 and -3 was due to the changes in their mRNAs. When confluent rabbit articular chondrocytes were treated with rhIL-1α (1 ng/mL) for 24 h, the expression of proMMPs-1 or -3 mRNA were significantly augmented (Fig. 4). Rhein (30 μM) down-regulated the expression of proMMP-1 and -3 mRNAs (50 and 63% inhibition, respectively). Dexamethasone (10 nM) also interfered with their expression (69 and 82% inhibition, respectively), but naproxen (1 μM) did not.

Discussion

Cartilage destruction is a crucial feature of OA and is generally considered irreversible. Accordingly, drugs that block the destruction of cartilage will be of therapeutic

Table I
Effects of rhein, naproxen and dexamethasone on the rhIL-1α-induced caseinolytic activity

Incubation time	Treatment	Conc. (μM)	Caseinolytic activity (units/mL)
24 h	None	—	0.45±0.05
	rhIL-1α	—	1.32±0.09[(a)]
	rhIL-1α+Rhein	3	1.25±0.06
	rhIL-1α+Rhein	10	1.13±0.26
	rhIL-1α+Rhein	30	0.98±0.06†
	rhIL-1α+Naproxen	1	1.21±0.14
	rhIL-1α+Dexamethasone	0.01	0.78±0.16[(c)]
48 h	None	—	0.65±0.27
	rhIL-1α	—	1.72±0.15[(b)]
	rhIL-1α+Rhein	3	1.80±0.12
	rhIL-1α+Rhein	10	1.59±0.11
	rhIL-1α+Rhein	30	1.33±0.08*
	rhIL-1α+Naproxen	1	1.69±0.11
	rhIL-1α+Dexamethasone	0.01	1.38±0.13[(d)]

Confluent chondrocytes in 100-mm dishes were treated with rhIL-1α (1 ng/mL) in the presence and absence of an indicated drug for 24 or 48 h. MMPs activities in the conditioned medium were determined using FITC-labeled casein as described in the text. Values represent means±S.D. for five independent experiments. [(a)] and [(b)], significantly different from untreated control group by Aspin–Welch test ($P<0.0001$ and $P=0.0001$, respectively). [(c)] and [(d)], significantly different from rhIL-1α-treated control group by Aspin–Welch test ($P=0.0002$ and $P=0.0050$, respectively). * and †, significantly different from rhIL-1α-treated control group by Dunnett's test ($P=0.0241$ and $P=0.0058$, respectively).

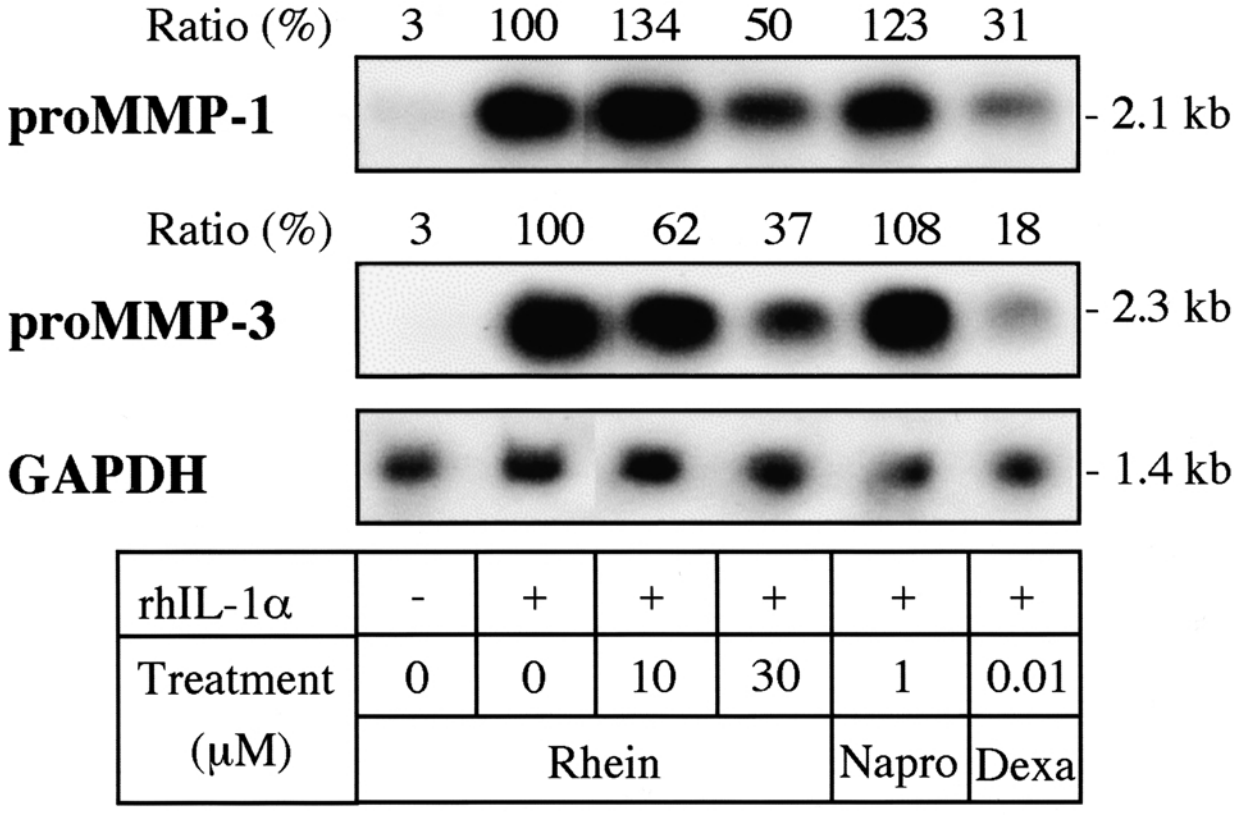

Fig. 4. Effects of rhein, naproxen and dexamethasone on the steady-state mRNA levels of proMMP-1, proMMP-3 and GAPDH. Confluent chondrocytes in 100-mm dishes were cultured with rhIL-1α (1 ng/mL) in the presence and absence of the indicated drug for 24 h. Total RNA (20 μg) was subjected to Northern blot analysis as described in the text. The same blot was stripped and reprobed with a GAPDH-encoding probe to confirm equal lane loadings. Two independent experiments using cells of different origins were highly reproducible, and representative data are shown. The relative amounts of proMMPs-1 and -3 mRNA were quantified by densitometric scanning and normalized to GAPDH mRNA by taking the rhIL-1α-treated cells as 100. Napro; naproxen and Dexa; dexamethasone.

value. Currently, the primary approach in the clinical treatment of OA involves the use of non-steroidal anti-inflammatory drugs (NSAIDs), analgesics and hyaluronan, which provide symptomatic relief but exert no apparent disease-modifying effect[35,36]. In some instances, NSAIDs may even be deleterious; they have been reported to inhibit the synthesis of aggregating proteoglycan, which plays a crucial role in maintaining the function of the cartilage[37]. Therefore, there is a critical need to develop alternative agents that prevent the destruction of cartilage and/or stimulate its proper repair.

Diacerein has proved to be effective and well tolerated in the long-term treatment of OA[15–17] and it is currently under evaluation as a disease modifying OA drug in randomized placebo-controlled clinical trials of patients with OA of the hip and knee. The efficacy of diacerein has been well documented in animal models, including the post-contusive rabbit model and the accelerated canine model of OA[18,19], but its precise mechanism of action on chondroprotection is unclear. To obtain further insight into the effects of diacerein, monolayer cultures of rabbit articular chondrocytes were used to investigate the effect of rhein, an active metabolite of diacerein, on the production of proMMPs and TIMP-1.

The activities of MMPs in tissues are regulated by a number of factors, including gene expression of proMMPs, extracelluar activation of proMMPs and inhibition of MMPs by their endogenous inhibitors of TIMPs. It is thought that much of the cartilage destruction seen in OA was found to be due to a local imbalance between activated MMPs and TIMPs[14]. In this communication, we have shown that rhein suppresses the rhIL-1α-induced production of proMMPs-1, -3, -9 and -13, along with a decrease in their mRNA expression. By contrast, rhein increased TIMP-1 production, suggesting that rhein reduces MMP activities at the inflammatory site; in fact rhein reduced the apparent caseinolytic activity in the culture media which precisely reflected the balance between MMPs and TIMPs. In addition, this is the first evidence that chondroprotective drugs including rhein and NSAIDs down-regulate the proMMP-13 production in articular chondrocytes.

The mechanisms by which rhein inhibits the production and gene expression of proMMPs are not clear at present. From this point of view, it is of interest that the up-regulation of collagenase gene expression by IL-1 is likely to be mediated by the transcription factors, c-fos and c-jun, and the activation of activator protein-1 (AP-1) activity[38]. In addition, it is also suggested that reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide, superoxide and nitric oxide act as intracellular signaling molecules mediating the biological effects of cytokines[39]. The blocking of the ROS production or decreasing ROS levels is known to reduce the IL-1β-induced c-fos and collagenase expression in chondrocytes[39]. The participation of intracelluar cAMP in the regulation of production of proMMPs including proMMPs-1, -3 and -9 is also reported in rabbit articular chondrocytes[26] and human synovial fibroblasts[40,41]. The actions of rhein and/or its mother compound of diacerein on the above signals are not fully understood; in bovine articular chondrocytes diacerein inhibits AP-1 activity and thereby results in the suppression of collagenase expression but the action of rhein is not specified[42]. Rhein is reported to reduce the production of superoxide anion in human neutrophils[23]. Taken together, it is likely that rhein modulates the production of proMMPs and TIMP-1 via interference with multiple signaling pathways in rabbit chondrocytes. Further studies are needed to elucidate the precise mechanism of action of rhein on the production of proMMPs and TIMPs in chondrocytes.

In conclusion, we have demonstrated for the first time that rhein, an active metabolite of diacerein down-regulates the gene-expression of proMMPs-1 and -3, and the production of proMMPs-1, -3, -9 and -13, and furthermore, it up-regulates the TIMP-1 production. The therapeutic effects of diacerein on OA and those observed in several animal models of OA may be due, at least in part, to the chondroprotective effect of rhein, its active metabolite.

References

1. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, *et al*. Matrix metalloproteinases: a review. Oral Biol Med 1993; 4:197–250.
2. Vincenti MP, Clark IM, Brinckerhoff CE. Using inhibitors of metalloproteinases to treat arthritis. Arthritis Rheum 1994;37:1115–26.
3. Zafarullah M, Pelletier JP, Cloutier JM, Martel-Pelletier J. Elevated metalloproteinase and tissue inhibitor of metalloproteinase mRNA in human osteoarthritic synovia. J Rheumatol 1993;20:693–7.
4. Mitchell PG, Magna HA, Reeves LM, Lopresti-Morrow LL, Yocum SA, Rosner PJ, *et al*. Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. J Clin Invest 1996;97:761–8.
5. Reboul P, Pelletier JP, Tardif G, Cloutier JM, Martel-Pelletier J. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. A role in osteoarthritis. J Clin Invest 1996;97:2011–19.
6. Okada Y, Shinmei M, Tanaka O, Naka K, Kimura A, Nakanishi I, *et al*. Localization of matrix metalloproteinase 3 (stromelysin) in osteoarthritic cartilage and synovium. Lab Invest 1992;66:680–90.
7. Murphy G, Cockett MI, Stephens PE, Smith BJ, Docherty AJ. Stromelysin is an activator of procollagenase. A study with natural and recombinant enzymes. Biochem J 1987;248:265–8.
8. Hembry RM, Bagga MR, Reynolds JJ, Hamblen DL. Immunolocalisation studies on six matrix metalloproteinases and their inhibitors, TIMP-1 and TIMP-2, in synovia from patients with osteo- and rheumatoid arthritis. Ann Rheum Dis 1995;54:25–32.
9. Ollivierre F, Gubler U, Towle CA, Laurencin C, Treadwell BV. Expression of IL-1 genes in human and bovine chondrocytes: a mechanism for autocrine control of cartilage matrix degradation. Biochem Biophys Res Commun 1986;141:904–11.
10. Arend WP, Dayer JM. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. Arthritis Rheum 1990;33:305–15.
11. Shinmei M, Masuda K, Kikuchi T, Shimomura Y. The role of cytokines in chondrocyte mediated cartilage degradation. J Rheumatol 1989;18:32–4.
12. Pelletier JP, McCollum R, Cloutier JM, Martel-Pelletier J. Synthesis of metalloproteases and interleukin 6 (IL-6) in human osteoarthritic synovial membrane is an IL-1 mediated process. J Rheumatol 1995;43: 109–14.
13. Cawston TE, Galloway WA, Mercer E, Murphy G, Reynolds JJ. Purification of rabbit bone inhibitor of collagenase. Biochem J 1981;195:159–65.

14. Dean DD, Martel-Pelletier J, Pelletier JP, Howell DS, Woessner JF Jr. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. J Clin Invest 1989;84:678–85.
15. Lingetti M, D'ambrosio PL, Grezia F, Sorrentino P, Lingetti E. A controlled study in the treatment of osteoarthritis with diacerhein (Artrodar). Curr Therp Res 1982;31:408–12.
16. Marcolongo R, Fioravanti A, Adami S, Tozzi E, Mian M, Zampieri A. Efficacy and tolerability of diacerhein in the treatment of osteoarthrosis. Curr Therp Res 1988;43:878–87.
17. Nguyen M, Dougados M, Berdah L, Amor B. Diacerhein in the treatment of osteoarthritis of the hip. Arthritis Rheum 1994;37:529–36.
18. Mazieres B, Berda L. Effect of diacerhein (ART 50) on an experimental post-contusive model of OA. Osteoarthritis Cart 1993;1:47.
19. Brandt KD, Smith G, Kang SY, Myers S, O'Connor B, Albrecht M. Effects of diacerhein in an accelerated canine model of osteoarthritis. Osteoarthritis Cart 1997;5:438–49.
20. Schongen RN, Giannetti BM, van de Leur E, Reinards R, Greiling H. Effect of diacetylrhein on the phagocytosis of polymorphonuclear leucocytes and its influence on the biosynthesis of hyaluronate in synovial cells. Arzneimittelforschung 1988;38:744–8.
21. Moore AR, Greenslade KJ, Alam CA, Willoughby DA. Effects of diacerhein on granuloma induced cartilage breakdown in the mouse. Osteoarthritis Cart 1998;6:19–23.
22. Debord P, Louchahi K, Tod M, Cournot A, Perret G, Petitjean O. Influence of renal function on the pharmacokinetics of diacerein after a single oral dose. Eur J Drug Metab Pharmacokinet 1994;19: 13–9.
23. Mian M, Brunelleschi S, Tarli S, Rubino A, Benetti D, Fantozzi R, *et al*. Rhein: an anthraquinone that modulates superoxide anion production from human neutrophils. J Pharm Pharmacol 1978;39:845–7.
24. Green WT Jr. Behavior of articular chondrocytes in cell culture. Clin Orthop 1971;75:248–60.
25. Nicolas P, Tod M, Padoin C, Petitjean O. Clinical pharmacokinetics of diacerein. Clin Pharmacokinet 1998;35:347–59.
26. Ito A, Nose T, Takahashi S, Mori Y. Cyclooxygenase inhibitors augment the production of pro-matrix metalloproteinase 9 (progelatinase B) in rabbit articular chondrocytes. FEBS Lett 1995;360:75–9.
27. Okada Y, Nagase H, Harris ED Jr. A metalloproteinase from human rheumatoid synovial fibroblasts that digests connective tissue matrix components. Purification and characterization. J Biol Chem 1986;261: 14245–55.
28. Okada Y, Takeuchi N, Tomita K, Nakanishi I, Nagase H. Immunolocalization of matrix metalloproteinase 3 (stromelysin) in rheumatoid synovioblasts (B cells): correlation with rheumatoid arthritis. Ann Rheum Dis 1989;48:645–53.
29. Vincenti MP, Coon CI, Mengshol JA, Yocum S, Mitchell P, Brinckerhoff CE. Cloning of the gene for interstitial collagenase-3 (matrix metalloproteinase-13) from rabbit synovial fibroblasts: differential expression with collagenase-1 (matrix metalloproteinase-1). Biochemical J 1998;331:341–6.
30. Ito A, Nagase H. Evidence that human rheumatoid synovial matrix metalloproteinase 3 is an endogenous activator of procollagenase. Arch Biochem Biophys 1988;267:211–16.
31. Hosono T, Ito A, Sato T, Nagase H, Mori Y. Translational augmentation of pro-matrix metalloproteinase 3 (prostromelysin 1) and tissue inhibitor of metalloproteinases (TIMP)-1 mRNAs induced by epidermal growth factor in human uterine cervical fibroblasts. FEBS Lett 1996;381:115–18.
32. Twining SS. Fluorescein isothiocyanate-labeled casein assay for proteolytic enzymes. Anal Biochem 1984;143:30–4.
33. Takahashi S, Ito A, Nagino M, Mori Y, Xie B, Nagase H. Cyclic adenosine 3′, 5′-monophosphate suppresses interleukin 1-induced synthesis of matrix metalloproteinases but not of tissue inhibitor of metalloproteinases in human uterine cervical fibroblasts. J Biol Chem 1991;266:19894–9.
34. Saus J, Quinones S, Otani Y, Nagase H, Harris ED Jr, Kurkinen M. The complete primary structure of human matrix metalloproteinase-3. Identity with stromelysin. J Biol Chem 1988;263:6742–5.
35. Bollet AJ. Analgesic and anti-inflammatory drugs in the therapy of osteoarthritis. Semin Arthritis Rheum 1981;11:130–2.
36. Pelletier JP, Martel-Pelletier J. Protective effects of corticosteroids on cartilage lesions and osteophyte formation in the Pond-Nuki dog model of osteoarthritis. Arthritis Rheum 1989;32:181–93.
37. Palmoski MJ, Brandt KD. Effect of salicylate on proteoglycan metabolism in normal canine articular cartilage in vitro. Arthritis Rheum 1979;22:746–54.
38. Lafyatis R, Kim SJ, Angel P, Roberts AB, Sporn MB, Karin M, et al. Interleukin-1 stimulates and all-trans-retinoic acid inhibits collagenase gene expression through its 5′ activator protein-1-binding site. Mol Endocrinol 1990;4:973–80.
39. Lo YY, Conquer JA, Grinstein S, Cruz TF. Interleukin-1β induction of c-fos and collagenase expression in articular chondrocytes: involvement of reactive oxygen species. J Cell Biochem 1998;69: 19–29.
40. Lane NE, Williams RJ 3rd, Schurman DJ, Smith RL. Inhibition of interleukin 1 induced chondrocyte protease activity by a corticosteroid and a nonsteroidal antiinflammatory drug. J Rheumatol 1992;19:135–9.
41. Smith RL, Kajiyama G, Lane NE. Nonsteroidal antiinflammatory drugs: effects on normal and interleukin 1 treated human articular chondrocyte metabolism in vitro. J Rheumatol 1995;22:1130–7.
42. Cruz TF, Tang J, Pronost S, Pujol JP. Molecular mechanisms implicated in the inhibition of collagenase expression by diacerheine. Rev Praticien 1996;46:S15–9.