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4′-Hydroxy aceclofenac suppresses the interleukin-1-induced production of promatrix metalloproteinases and release of sulfated-glycosaminoglycans from rabbit articular chondrocytes

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Abstract

This study demonstrates the novel actions of a non-steroidal anti-inflammatory drug aceclofenac, which is frequently used for rheumatoid arthritis and osteoarthritis. 4′-Hydroxy aceclofenac, a main metabolite of aceclofenac in humans, down-regulated the production of promatrix metalloproteinase-1/procollagenase 1 and promatrix metalloproteinase-3/prostromelysin 1 along with a decrease in their mRNAs in rabbit articular chondrocytes and synoviocytes, and interfered with the release of sulfated-glycosaminoglycans (proteoglycans) from the chondrocytes. 4′-Hydroxy aceclofenac also suppressed the proliferation of rabbit synoviocytes. In contrast, aceclofenac itself and its other metabolites, diclofenac and 4′-hydroxy declofenac, did not exert obvious actions on cellular functions. Therefore, it is suggested that the therapeutic effects of aceclofenac on rheumatoid arthritis and osteoarthrits are, at least in part, due to the novel chondroprotective effect of 4′-hydroxy aceclofenac via the suppression of promatrix metalloproteinase production and proteoglycan release. There is also evidence that inhibition of synoviocyte proliferation and the known inhibitory action on prostaglandin E_2 production play a role.

Keywords: Osteoarthritis; Rheumatoid arthritis; Aceclofenac; 4′-Hydroxy aceclofenac; Pro-matrix metalloproteinase 1; Pro-matrix metalloproteinase 3; Articular chondrocyte, rabbit

1. Introduction

Aceclofenac, 2-[(2, 6-dichlorophenyl)amino] phenylacetoxyacetic acid, is a novel non-steroidal anti-inflammatory drug (NSAID) developed in Spain (Grau et al., 1991) and its biological actions are of interest: it has effective therapeutic actions on rheumatoid arthritis and osteoarthritis (Ballesteros et al., 1990; Ward et al., 1995), reduces prostaglandin E_2 levels in the synovial fluid of patients with acute knee pain (Cecchettin et al., 1988) and suppresses prostaglandin E_2 production by blood polymorphonuclear leukocytes or mononuclear cells from patients with severe osteoarthritis (Gonzalez et al., 1994). Our recent study also demonstrated that aceclofenac suppresses the production of prostaglandin E_2 by rheumatoid synovial cells in vitro (Yamazaki et al., 1997), but the inhibition of prostaglandin E_2 does not satisfactorily explain the therapeutic actions of aceclofenac, and therefore the exact mechanism of aceclofenac on rheumatoid arthritis and osteoarthritis is not yet fully understood.

The destruction of connective tissue matrix components in rheumatoid arthritis and osteoarthritis is a serious condition and causes the impairment of joint function. Matrix metalloproteinases are considered to play a central role in these processes (for review; Nagase and Okada, 1996). In particular, matrix metalloproteinase-1/collagenase 1, which specifically degrades native types I, II and III collagen, and matrix metalloproteinase-3/stromelysin 1, which digests proteoglycans and collagen types IX and X, are probably key enzymes for the pathological destruction of cartilage (Nagase and Okada, 1996). In fact, high levels of matrix metalloproteinases are found in the serum and synovial fluid of patients with rheumatoid arthritis and osteoarthritis (Arend and Dayer, 1990; Walakovits et al.,

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1992; Tetlow et al., 1993) and are secreted from rheumatoid synovial cells (Dayer et al., 1976; Okada et al., 1986, 1990). In articular chondrocytes and synovial cells, interleukin 1 is known to induce and/or augment the production of promatrix metalloproteinases-1 and -3 as well as prostaglandin E_2. It was recently reported that prostaglandin E, an inflammatory mediator, suppresses the production of promatrix metalloproteinase-1 in human fibroblasts and rabbit synovial cells along with an increase in intracellular 3′, 5′-cyclic AMP (Case et al., 1990; Salvatori et al., 1992). Thus, the question of whether prostaglandin E is an inflammatory or anti-inflammatory agent in osteoarthritis and rheumatoid arthritis is of interest.

In order to clarify the potential action mechanism of aceclofenac in vivo on rheumatoid arthritis and/or osteoarthritis, we examined the effects of aceclofenac and its metabolites on the production of promatrix metalloproteinases-1 and -3 as well as prostaglandin E_2 in rabbit articular chondrocytes and synovial cells. We found that 4′-hydroxy aceclofenac, one of the main metabolites of aceclofenac in humans (Bort et al., 1996), interfered with the interleukin-1-mediated production of promatrix metalloproteinases-1 and -3 and the release of proteoglycans from rabbit chondrocytes, and thus participates in chondroprotection. Therefore, aceclofenac is classified as a novel NSAID which simultaneously down-regulates the production of promatrix metalloproteinases as well as prostaglandin E_2 in osteoarthritis and/or rheumatoid arthritis.

2. Materials and methods

2.1. Materials

Aceclofenac was kindly supplied by Almirall Prodesfarma (Barcelona, Spain). Diclofenac sodium salt (diclofenac), indomethacin and dexamethasone were purchased from Sigma (St. Louis, MO, USA). 4′-Hydroxy aceclofenac and 4′-hydroxy diclofenac were chemically synthesized by Teikoku Hormone Manufacturing (Tokyo, Japan). Sheep anti-(rabbit promatrix metalloproteinase-1) and sheep anti-(rabbit promatrix metalloproteinase-3) antiserum, human matrix metalloproteinase-1 cDNA (1.6 kb) and human matrix metalloproteinase-3 cDNA (1.4 kb) were kindly provided by Dr. H. Nagase of the Kennedy Institute of Rheumatology, Imperial College, London, UK. Human recombinant interleukin-1α (2×10^7 units/mg) was generously donated by Dainippon Pharmaceutical (Osaka, Japan). Hydroxamate matrix metalloproteinase inhibitor, GM6001-X ($HONHCOCH_2CH$(isobutyl)CO–Try(OMe)–NHMe) (Grobelny et al., 1992) was a generous gift from Dr. J. Oloksyszyn of Osteo-Arthritis Sciences. The following reagents were obtained commercially: lactalbumin hydrolysate, collagenase type I and alkaline phosphatase-conjugated anti-sheep Immunoglobulin G antibody (Sigma); Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Grand Island, NY, USA), and fetal bovine serum (Whittaker Bioproducts, Walkerville, MD, USA). Other reagents used were those described in previous reports (Ito et al., 1995; Takahashi et al., 1991).

2.2. Cell culture of rabbit articular chondrocytes and synovial fibroblasts

Rabbit synovial fibroblasts and articular chondrocytes were prepared from Japanese white rabbits (female, weighing 0.35–0.4 kg) as described previously (Ito et al., 1995). The cells were maintained in 10% fetal bovine serum/DMEM/antibiotics until confluence. In all experiments, articular chondrocytes at the primary culture and synovial cells up to the 7th passage were used, respectively. To estimate the production of promatrix metalloproteinases and prostaglandin E_2, the culture medium was changed to DMEM/0.2% lactalbumin hydrolysate after confluence, and aceclofenac and other reagents were added to the medium as an ethanol solution. The concentrations of the reagents used reflected levels that correspond to serum levels obtained in phase I studies (R. Yamazaki et al. unpublished data). The final ethanol concentration was 0.1% in all cultures, and the same amount of vehicle was added to the control cultures. The harvested culture media were stored at -20°C until use.

2.3. Western blot analysis

Promatrix metalloproteinases in the culture medium were analyzed by Western blot analysis. The sample (1 ml) from triplicate wells was concentrated with trichloroacetic acid (final concentration of 3.3%). The resultant precipitates were dissolved in 20 μl of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample solution and then a portion of the solution was subjected to SDS-PAGE with 10% acrylamide gel under reducing conditions (Laemmli, 1970). After electrophoresis, proteins in the gel were electrotransferred onto a nitrocellulose filter. The filter was reacted with the respective antibody, which was then complexed with the alkaline phosphatase-conjugated second antibody. Immunoreactive promatrix metalloproteinases were visualized indirectly with 5-bromo-4-chloro-3-indolyl phosphate and Nitro Blue Tetrazolium as described previously (Takahashi et al., 1991). The amount of promatrix metalloproteinases was determined by densitometric analysis.

2.4. RNA extraction and Northern blot analysis

Confluent rabbit cells in 60-mm diameter dishes were treated with interleukin-1α and/or respective reagent. After 24 h, total cytoplasmic RNA was extracted with Isogen (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. Then the extracted RNA (15 μg) was denatured with formamide and formaldehyde, electrophoresed on 1% agarose gel containing 1×3(*N*-mor-

pholino)propanesulfonic acid (MOPS) and 2.2 M formaldehyde, and transferred onto a nylon membrane in 10 × standard saline citrate (SSC). The membrane was hybridized with ^{32}P-labeled random primed human promatrix metalloproteinase-1 or promatrix metalloproteinase-3 cDNA in 50% formamide/5 × SSC/0.1% SDS/1 × Denhardt's solution/50 mM Na_3PO_4/200 μg/ml of heat-denatured salmon sperm DNA at 42°C for 18 h. After hybridization, the membrane was washed in 1 × SSC/0.1% SDS and then in 0.1 × SDS/0.1% SDS at room temperature for 30 min, and then exposed to Konica X-ray film (Tokyo, Japan) at −80°C. The expression of mRNA was quantified by densitometric scanning of the autoradiograms.

2.5. Measurement of release of sulfated glycosaminoglycans from cultured chondrocytes

Confluent rabbit articular chondrocytes in 48-multiwell plates were treated with interleukin-1α and/or each reagent for 24 h, and the culture media and cell layer were collected separately. The cells were first treated with 0.03% papain at 65°C for 6 h, and the resultant sulfated glycosaminoglycans (proteoglycans) in the digest were determined with dimethylmethylene blue by the method of Farndale et al. (1982). The sulfated glycosaminoglycans in the culture media were directly assayed with dimethylmethylene blue. The release of sulfated glycosaminoglycans is expressed as the percentage of that released into the medium fraction.

2.6. Assay for cell proliferation

Cell proliferation was monitored by WST-1 assay using sulfonated tetrazolium salt, 4-[3-(4-indophenyl1)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate (Ishiyama et al., 1993).

3. Results

3.1. Effect of aceclofenac and its metabolites on the production of promatrix metalloproteinases-1 and -3 in rabbit chondrocytes and synovial fibroblasts

We first examined whether aceclofenac and its metabolites in humans suppress the production of promatrix metalloproteinases which closely participate in the destruction

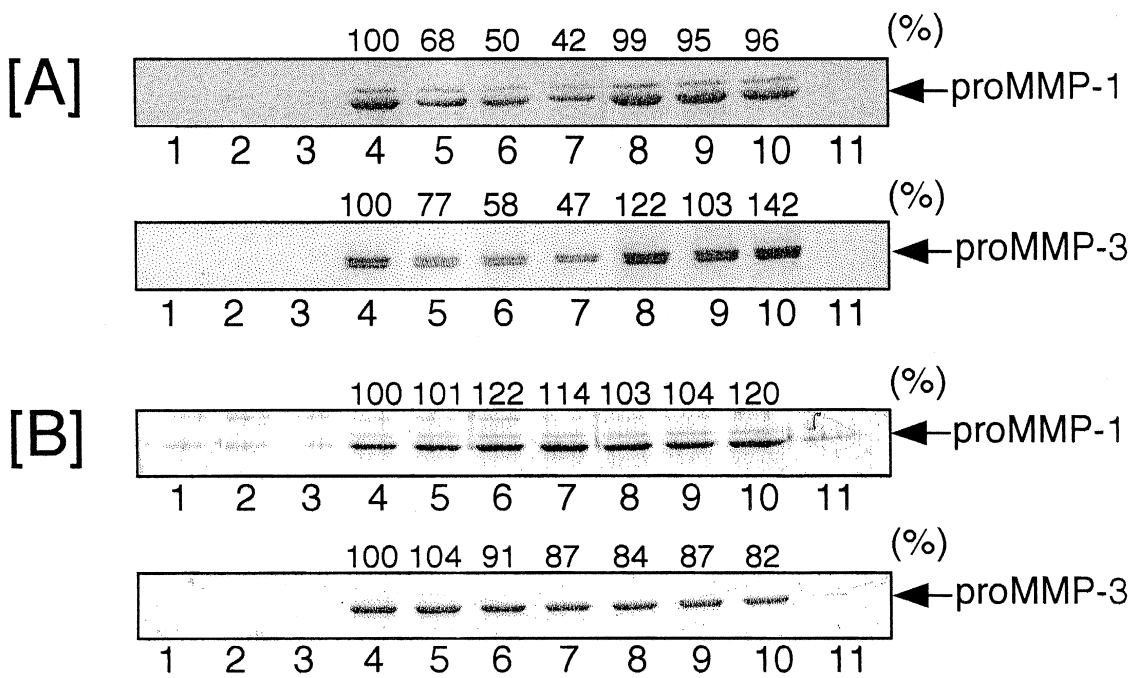

Fig. 1. Effects of aceclofenac and its metabolites on interleukin-1α-induced production of promatrix metalloproteinases-1 and -3 in rabbit articular chondrocytes. Confluent rabbit chondrocytes in 24-multiwell plates were treated with interleukin-1α (1 ng/ml), aceclofenac and/or its metabolites in 1.0 ml of DMEM/0.2 % lactalbumin hydrolysate for 24 h. The harvested and mixed culture media from triplicate wells were concentrated and subjected to Western blot analysis for promatrix metalloproteinases-1 and -3. Three independent experiments using cells of different origins were highly reproducible, and typical data are shown. The relative amounts of promatrix metalloproteinases-1 and -3 were quantified by densitometric scanning, taking the interleukin-1α-treated cells as 100%. Panel [A]: effects of aceclofenac and 4′-hydroxy aceclofenac. Lane 1, non-treated control; lane 2, 4′-hydroxy aceclofenac (30 μM); lane 3, aceclofenac (30 μM); lane 4, interleukin-1α; lanes 5–7, interleukin-1α plus 4′-hydroxy aceclofenac (3, 15 and 30 μM, respectively); lanes 8–10, interleukin-1α plus aceclofenac (3, 15 and 30 μM, respectively) and lane 11, interleukin-1α plus dexamethasone (1 μM). Panel [B]: effects of diclofenac and 4′-hydroxy diclofenac. Lane 1, non-treated control; lane 2, 4′-hydroxy diclofenac (30 μM); lane 3, diclofenac (30 μM); lane 4, interleukin-1α; lanes 5–7, interleukin-1α plus 4′-hydroxy diclofenac (3, 15 and 30 μM, respectively); lanes 8–10, interleukin-1α plus diclofenac (3, 15 and 30 μM, respectively) and lane 11, interleukin-1α plus dexamethasone (1 μM).

of articular cartilage in osteoarthritis or rheumatoid arthritis. When rabbit articular chondrocytes were treated with interleukin-1α, the production of promatrix metalloproteinases-1 and -3 was clearly augmented as compared to the control (Fig. 1A and B, lane 4). 4′-Hydroxy aceclofenac, one of the main metabolites in humans (Bort et al., 1996), suppressed the interleukin-1α-induced production of promatrix metalloproteinases-1 and -3 in a dose-dependent manner (3–30 μM); approximately 50% of promatrix metalloproteinases-1 and -3 production was inhibited by 4′-hydroxy aceclofenac at 30 μM (Fig. 1A, lane 7). Dexamethasone (1 μM), a well-characterized suppressor of promatrix metalloproteinase production in most cell species, completely suppressed the interleukin-1α-mediated production of matrix metalloproteinases-1 and -3 (Fig. 1A and B, lane 11). Aceclofenac, diclofenac and 4′-hydroxy diclofenac did not exert any effects on their production. These four reagents, however, did not modify the production of tissue inhibitor of metalloproteinase-1 (TIMP-1) in rabbit articular chondrocytes (data not shown). A very similar suppressive action of 4′-hydroxy aceclofenac, but not the other reagents, was observed with rabbit synovial fibroblasts (Fig. 2), supporting that 4′-hydroxy aceclofenac predominantly suppresses the production of promatrix metalloproteinases-1 and -3.

To further examine whether the decrease in promatrix metalloproteinases-1 and -3 levels obtained with 4′-hydroxy aceclofenac was due to changes in their mRNAs, we assessed changes in the steady-state levels of mRNAs for promatrix metalloproteinases-1 and -3 by Northern blot analysis. As shown in Fig. 3, non-treated control cells contained low levels of transcripts for promatrix metalloproteinases-1 and -3, but interleukin-1α clearly increased their transcripts. 4′-Hydroxy aceclofenac decreased the interleukin-1α-augmented expression of steady-state levels of their mRNAs. These results indicate that 4′-hydroxy aceclofenac down-regulates the production of promatrix metalloproteinases-1 and -3 in rabbit articular chondrocytes and synovial cells at the transcriptional level.

3.2. Effect of aceclofenac and its metabolites on the release of sulfated glycosaminoglycans from rabbit articular chondrocytes

When confluent rabbit articular chondrocytes were treated with interleukin-1α, the amount of sulfated gly-

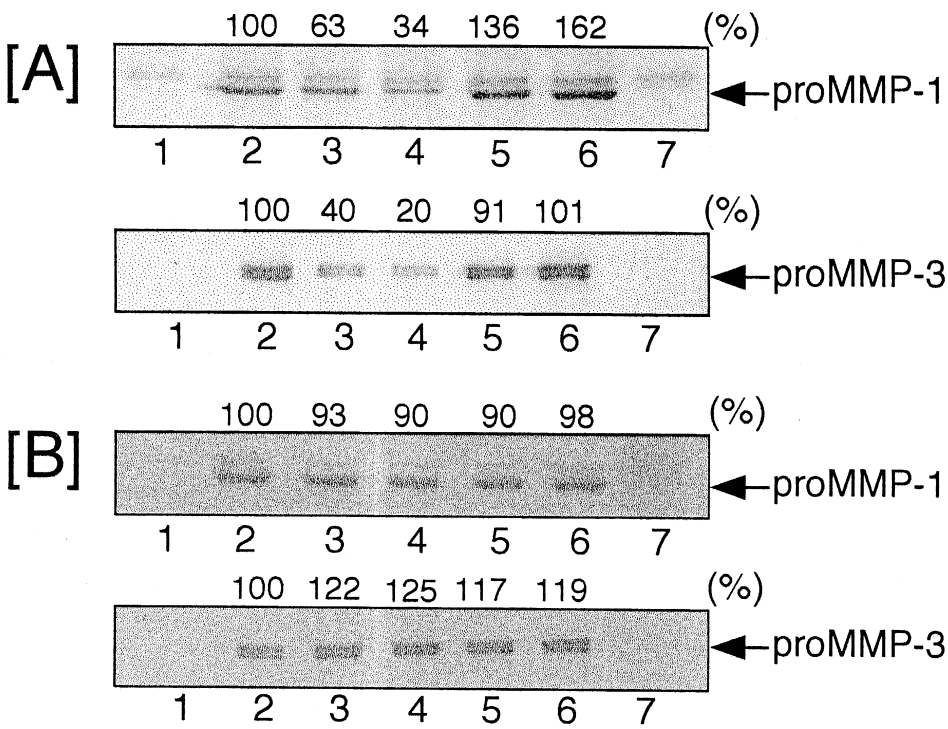

Fig. 2. Effects of aceclofenac and its metabolites on interleukin-1α-induced production of promatrix metalloproteinases-1 and -3 in rabbit synovial fibroblasts. Confluent rabbit synoviocytes at the fourth passage in 24-multiwell plates were treated with interleukin-1α (1 ng/ml), aceclofenac and/or its metabolites in 1.0 ml of DMEM/0.2 % lactalbumin hydrolysate for 24 h. Then the amount of promatrix metalloproteinases-1 and -3 in the culture media and their relative amounts were assessed by Western blot analysis and densitometric scanning, as decribed in Fig. 1. Three independent experiments using cells of different origins were highly reproducible, and typical data are shown. Panel [A]: effects of aceclofenac and 4′-hydroxy aceclofenac. Lane 1, non-treated control; lane 2, interleukin-1α; lanes 3 and 4, interleukin-1α plus 4′-hydroxy aceclofenac (3 and 15 μM); lanes 5 and 6, interleukin-1α plus aceclofenac (3 and 15 μM) and lane 7, interleukin-1α plus dexamethasone (1 μM). Panel [B]: effects of diclofenac and 4′-hydroxy diclofenac. Lane 1, non-treated control; lane 2, interleukin-1α; lanes 3 and 4, interleukin-1α plus 4′-hydroxy diclofenac (3 and 15 μM); lanes 5 and 6, interleukin-1α plus diclofenac (3 and 15 μM) and lane 7, interleukin-1α plus dexamethasone (1 μM).

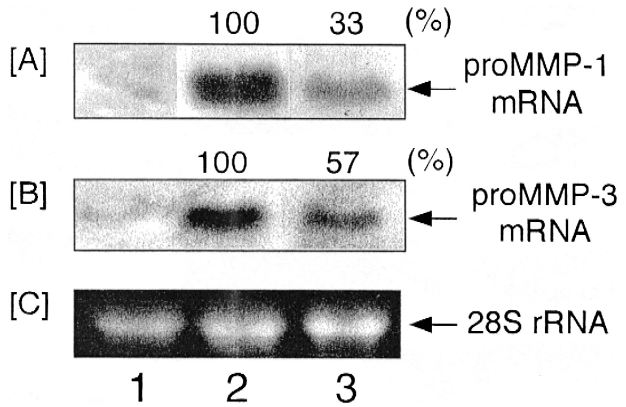

Fig. 3. 4′-Hydroxy aceclofenac suppressed the expression of genes for promatrix metalloproteinases-1 and -3 in rabbit articular chondrocytes. Confluent chondrocytes in 60-mm diameter dishes were treated with interleukin-1α (1 ng/ml) and/or 4′-hydroxy aceclofenac for 24 h in 5 ml of DMEM/0.02 % lactalbumin hydrolysate. Promatrix metalloproteinases-1 and -3 mRNAs in total cytoplasmic RNA (15 μg) were monitored by Northern blot analysis as described in the text. The relative levels of their mRNAs were quantified by densitometric scanning and normalized to 28S rRNA, taking the untreated control cells as 100 %. Experiments using cells of different origins were conducted in duplicate and a typical set of data is shown. Lane 1, non-treated control; lane 2, interleukin-1α and lane 3, interleukin-1α plus 4′-hydroxy aceclofenac (30 μM).

cosaminoglycans (proteoglycans) released in the culture medium was significantly greater (about 1.5-fold) than that of the control, as shown in Fig. 4A. This interleukin-1α-augmented release of sulfated glycosaminoglycans was almost completely inhibited by a matrix metalloproteinase inhibitor, GM6001-X (Grobelny et al., 1992) (Fig. 4B), indicating that active matrix metalloproteinases and/or aggrecanase (Lark et al., 1997; Tortorella et al., 1999) are likely to closely participate in the release of sulfated glycosaminoglycans from rabbit chondrocytes. 4′-Hydroxy aceclofenac suppressed the interleukin-1α-mediated release of sulfated glycosaminoglycans from chondrocytes; most of the interleukin-1α-induced release was inhibited by 15 μM of 4′-hydroxy aceclofenac. In contrast aceclofenac itself, and the other metabolites, diclofenac and 4′-hydroxy diclofenac, did not show any effects (Fig. 4A).

3.3. Aceclofenac and 4′-hydroxy aceclofenac interfere with the proliferation of rabbit synovial fibroblasts

We further examined whether aceclofenac affected the proliferation of rabbit synovial fibroblasts, which is likely to be related to the formation of pannus in rheumatoid arthritis. When rabbit synovial cells in the growth phase were treated with interleukin-1α, their proliferation was slightly, but steadily, promoted (1.3-fold) as compared to that of control cells (Fig. 5). 4′-Hydroxy aceclofenac was found to interfere with the interleukin-1α-mediated proliferation of the cells. Aceclofenac (30 μM) also suppressed the proliferation of synovial cells in the growth phase. In contrast, diclofenac and 4′-hydroxy diclofenac further augmented the interleukin-1α-mediated proliferation of rabbit synovial cells. This coincides with the previous observa-

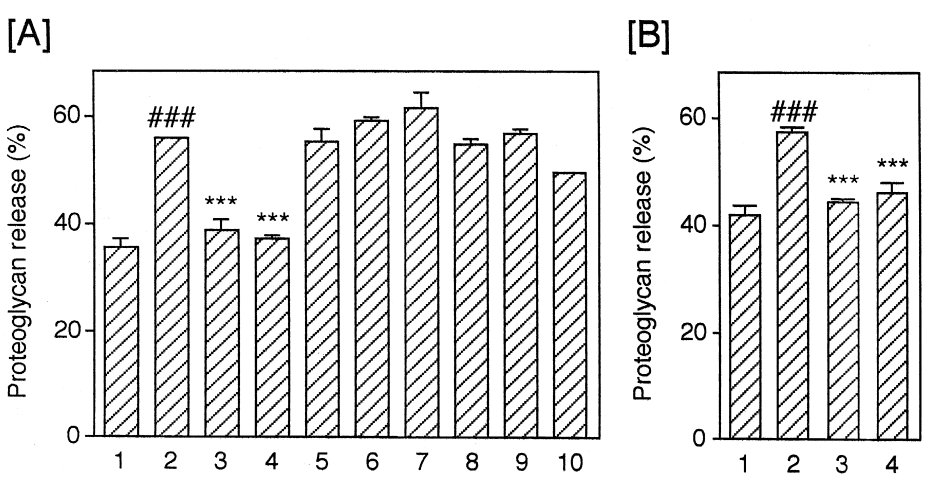

Fig. 4. Effects of aceclofenac and its metabolites on the release of sulfated glycosaminoglycans from cultured rabbit articular chondrocytes. Confluent rabbit chondrocytes in 24-multiwell plates were treated with interleukin-1α (1 ng/ml), aceclofenac, its metabolites and/or GM6001-X at indicated concentrations in 1 ml of DMEM/0.2% lactalbumin hydrolysate for 24 h. Sulfated glycosaminoglycans (proteoglycans) in the cells and medium were determined by using dimethylmethylene blue reagent as described in the text, and the release of proteoglycans was expressed as the percentage of proteoglycans released into the medium fraction. Data are the means ± SD for triplicate wells. ### and ***, significantly different from non-treated control cells and from interleukin-1α-treated cells ($P < 0.001$), respectively, as determined by Student's *t*-test. Three independent experiments using cells of different origins were highly reproducible, and typical data are shown. Panel [A]: effects of aceclofenac and its metabolites on the release of proteoglycans. Lane 1, non-treated control; lane 2, interleukin-1α; lanes 3 and 4, interleukin-1α plus 4′-hydroxy aceclofenac (15 and 30 μM); lanes 5 and 6, interleukin-1α plus aceclofenac (15 and 30 μM); lanes 7 and 8; interleukin-1α plus 4′-hydroxy diclofenac (15 and 30 μM); lanes 9 and 10; interleukin-1α plus diclofenac (15 and 30 μM). The amount of proteoglycans released from control and IL-1α-treated cells was 28.2 ± 5.5 and 52.4 ± 0.5 (μg/24 h), respectively. Panel [B]: effect of the matrix metalloproteinase inhibitor GM6001-X on the release of proteoglycans. Lane 1, non-treated control; lane 2, interleukin-1α; lanes 3, interleukin-1α plus GM6001-X (10 μM) and lane 4, interleukin-1α plus 4′-hydroxy aceclofenac (30 μM).

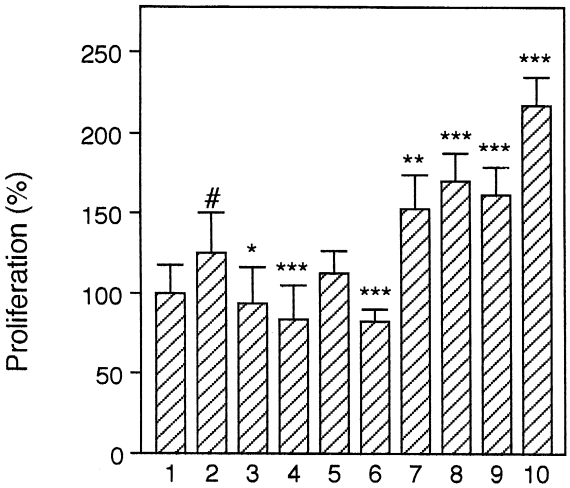

Fig. 5. Effects of aceclofenac and its metabolites on the proliferation of rabbit synovial fibroblasts. Rabbit synoviocytes at the 4th passage suspended at 2×10^3 cells/100 μl of 10% fetal bovine serum/DMEM were seeded in 96-multiwell plates and allowed to adhere for 2 h and then treated with interleukin-1α (1 ng/ml), aceclofenac and/or its metabolites in the same culture medium for 48 h. Cellular proliferation was assessed by WST-1 assay. Three independent experiments using cells of different origins were highly reproducible, and typical data are shown. Lane 1, non-treated control; lane 2, interleukin-1α; lanes 3 and 4, interleukin-1α plus 4′-hydroxy aceclofenac (7.5 and 30 μM); lanes 5 and 6, interleukin-1α plus aceclofenac (7.5 and 30 μM); lanes 7 and 8, interleukin-1α plus 4′-hydroxy diclofenac (7.5 and 30 μM) and lanes 9 and 10, interleukin-1α plus diclofenac (7.5 and 30 μM). Data are the means ± SD of six wells. #, significantly different from non-treated control cells ($P < 0.05$); *, ** and ***, significantly different from interleukin-1α-treated cells ($P <$ 0.05, 0.001 and 0.005, respectively) as determined by Student's *t*-test.

tion that indomethacin further enhances the interleukin 1-mediated proliferation of synovial cells (Kumkumian et al., 1989). Therefore, the effect of 4′-hydroxy aceclofenac and aceclofenac on cell growth is clearly distinct from that of 4′-hydroxy diclofenac and diclofenac.

4. Discussion

NSAIDs are primarily chosen to reduce the production of the inflammatory mediator prostaglandin E_2 in osteoarthritis and/or rheumatoid arthritis. It is, however, reported that the inhibiting activity of NSAIDs on prostaglandin E_2 production is not related to the improvement of osteoarthritis symptoms (Rashad et al., 1989). Therefore, the desirable therapeutic effects of aceclofenac on osteoarthritis and rheumatoid arthritis (Ballesteros et al., 1990; Ward et al., 1995) are not fully explained by the suppression of prostaglandin E_2 production (Yamazaki et al., 1997). We have examined the effects of aceclofenac and its metabolites on the interleukin-1α-induced production of promatrix metalloproteinases-1 and -3 in rabbit articular chondrocytes and synoviocytes, and found that 4′-hydroxy aceclofenac, a main metabolite of aceclofenac in humans, effectively interfered with the interleukin-1α-induced production of promatrix metalloproteinases-1 and -3, accompanying the decrease in the steady-state levels of their mRNAs. In contrast, 4′-hydroxy acceclofenac as well as aceclofenac, 4′-hydroxy diclofenac and diclofenac did not modulate the production of tissue inhibitor of metalloproteinases-1, indicating that suppression by 4′-hydoxy aceclofenac of the production of promatrix metalloproteinases-1 and -3 is the result of a novel, but not cytotoxic action of 4′-hydroxy aceclofenac.

Next, we examined the effect of 4′-hydroxy aceclofenac on the release of sulfated glycosaminoglycans, which is likely to reflect proteoglycan release from chondrocytes. Tortorella et al. (1999) recently reported that the release of proteoglycans from cartilage is mainly mediated by aggrecanase and that a hydroxamate matrix metalloproteinase inhibitor, BB-94 effectively inhibits the activity of aggrecanase. We also demonstrated that a synthetic hydroxamate matrix metalloproteinase inhibitor GM6001-X (Grobelny et al., 1992) completely inhibited the interleukin-1α-induced release of sulfated glycosaminoglycans from rabbit chondrocytes. It is, therefore, very likely that 4′-hydroxy aceclofenac inhibits the production of aggrecanase as well as matrix metalloproteinases-1 and -3 in rabbit chondrocytes.

The reason why 4′-hydroxy aceclofenac effectively suppresses the production of promatrix metalloproteinases-1 and -3 in rabbit chondrocytes and synovial fibroblasts is not well understood. In this respect, the fact that prostaglandin E reduces the production of promatrix metalloproteinases and indomethacin augments their production in human and rabbit fibroblasts and chondrocytes is of interest (Case et al., 1990; Salvatori et al., 1992; Ito et al., 1995). Furthermore, prostaglandin E suppresses the proliferation of synovial cells (Kumkumian et al., 1989) and T-cells (Knudsen et al., 1986), and the production of interleukin-1 in macrophages (Kunkel and Chensue, 1985; Funaki et al., 1992). Prostaglandin E augments the accumulation of intracellular 3′, 5′-cyclic AMP, which eventually suppresses the production of promatrix metalloproteinases in rabbit articular chondrocytes (Ito et al., 1995). We have, however, recently confirmed that 4′-hydroxy aceclofenac inhibits the production of prostaglandin E_2 in rabbit synovial fibroblasts and chondrocytes as well as in rheumatoid synovial cells (Yamazaki et al., 1997) and does not increase the intracellular 3′, 5′-cyclic AMP level in rabbit synovial cells (H. Akimoto and A. Ito, unpublished data). It is highly possible that 4′-hydroxy aceclofenac suppresses the production of promatrix metalloproteinases in synoviocytes and chondrocytes independently of a reduction in prostaglandin E_2 and/or increase in intracellular 3′, 5′-cyclic AMP. 4′-Hydroxy aceclofenac might interfere with the production of promatrix metalloproteinases via direct suppression of intracellular signaling and/or gene expression of matrix metalloproteinases. Both 4′-hydroxy aceclofenac and aceclofenac, but not diclofenac and 4′-hydroxy diclofenac, suppressed the proliferation of

rabbit synovial cells, suggesting that these compounds may have the potential to interfere with the proliferation of the rheumatoid synovial membrane. However, the reason why both 4′-hydroxy aceclofenac and aceclofenac exerted similar anti-proliferative effects on synovial cells is unclear at present. The action mechanisms of 4′-hydroxy aceclofenac on the production of promatrix metalloproteinases and on the proliferation of synovial cells might be different. The exact mechanism of action of 4′-hydroxy aceclofenac should be clarified in the future.

In conclusion, we have demonstrated that 4′-hydroxy aceclofenac, a main metabolite of aceclofenac in humans, suppresses the interleukin-1-induced production of promatrix metalloproteinases and release of proteoglycans from rabbit chondrocytes and interferes with the proliferation of synoviocytes. Therefore, it is very likely that the therapeutic effects of aceclofenac in rheumatoid arthritis and/or osteoarthritis are, at least in part, due to the novel chondroprotective effects of 4′-hydroxy aceclofenac along with the known inhibitory action on the prostaglandin E_2 production (Yamazaki et al., 1997).

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