Hydrolytic Activity Is Essential for Aceclofenac To Inhibit Cyclooxygenase in Rheumatoid Synovial Cells

RYUTA YAMAZAKI, SHINICHI KAWAI, TSUNEIO MATSUMOTO, TAKESHI MATSUZAKI, SHUSUKE HASHIMOTO, TERUO YOKOKURA, RENZO OKAMOTO, TOMHIISA KOSHINO, and YUTAKA MIZUSHIMA

Yakult Central Institute for Microbiological Research, Kunitachi-shi, Tokyo (R.Y., T.M., T.M., S.H., T.Y.); Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki-shi, Kanagawa (S.K., Y.M.); and Department of Orthopaedic Surgery, Yokohama City University School of Medicine, Yokohama-shi, Kanagawa (R.O., T.K.), Japan

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ABSTRACT

To investigate the mechanisms of action underlying the anti-inflammatory effects of the nonsteroidal anti-inflammatory drug aceclofenac in humans, we studied the metabolism of aceclofenac in detail in primary cultured synovial cells of 10 patients with rheumatoid arthritis. Aceclofenac and 4'-hydroxyaceclofenac are the major compounds in human blood after the administration of aceclofenac, but they had no inhibitory effects on cyclooxygenase (COX) activity or COX expression in the rheumatoid synovial cells. In contrast, aceclofenac and 4'-hydroxyaceclofenac reduced prostaglandin E₂ (PGE₂) production by the rheumatoid synovial cells. We also observed that aceclofenac and 4'-hydroxyaceclofenac were hydrolyzed into the COX inhibitors diclofenac and 4'-hydroxydiclofenac, respectively, by the rheumatoid synovial cells. However, the hydrolytic activity differed markedly among the cell preparations. Because the suppressive potency of aceclofenac and 4'-hydroxyaceclofenac against the PGE₂ production was proportionately correlated with the hydrolytic activity in rheumatoid synovial cell preparations, we suggest that the suppressive effects of aceclofenac and 4'-hydroxyaceclofenac on PGE₂ production are facilitated by the hydrolytic activity in rheumatoid synovial cells.

Prostaglandin E₂ (PGE₂) is an important mediator of the pain and edema associated with rheumatoid synovitis in rheumatoid arthritis (Robinson et al., 1975; Dayer et al., 1977). Nonsteroidal anti-inflammatory drugs (NSAIDs) have generally been demonstrated to inhibit cyclooxygenase (COX) activity and to suppress the PGE₂ production by inflammatory cells, which are likely to be a primary source of PGE₂ (Vane, 1971). NSAIDs are thus frequently used extensively in the treatment of rheumatoid arthritis.

Aceclofenac, 2-[(2, 6-dichlorophenyl) amino] phenylacetoxyacetic acid, is a novel NSAID developed in Spain (Grau et al., 1991a,b). Aceclofenac has been shown to have marked therapeutic effects on rheumatoid arthritis and osteoarthritis and a good level of tolerability (Ballesteros et al., 1990; Dayer et al., 1991a,b). Aceclofenac has been shown to have marked therapeutic effects on rheumatoid arthritis and osteoarthritis, which are likely to be a primary source of PGE₂ (Vane, 1971). NSAIDs are thus frequently used extensively in the treatment of rheumatoid arthritis.

Aceclofenac and 4'-hydroxyaceclofenac were hydrolyzed into the COX inhibitors diclofenac and 4'-hydroxydiclofenac, respectively, by the rheumatoid synovial cells. However, the hydrolytic activity differed markedly among the cell preparations. Because the suppressive potency of aceclofenac and 4'-hydroxyaceclofenac against the PGE₂ production was proportionately correlated with the hydrolytic activity in rheumatoid synovial cell preparations, we suggest that the suppressive effects of aceclofenac and 4'-hydroxyaceclofenac on PGE₂ production are facilitated by the hydrolytic activity in rheumatoid synovial cells.

Experimental Procedures

Materials. Aceclofenac was supplied by Almirall Prodesfarma (Barcelona, Spain). Diclofenac sodium (diclofenac), indomethacin, and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO). 4'-Hydroxyaceclofenac and 4'-hydroxydiclofenac were chemically synthesized by Teikoku Hormone Manufacturing (Tokyo, Japan). These drugs were dissolved in dimethyl sulfoxide before use. Other materials were purchased from the following sources: rabbit

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ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; PGE₂, prostaglandin E₂; PMN, polymorphonuclear leukocyte; FCS, fetal calf serum; IL-1β, interleukin-1β; CRP, C-reactive protein.
anti-COX-1 antisera (Oxford Biomedical Research, Oxford, UK); rabbit anti-human COX-2 antisera and the PGE_2 enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI); arachidonic acid, collagenase type I, and alkaline phosphatase-conjugated anti-rabbit IgG antibody (Sigma); Dulbecco’s modified Eagle’s medium and serum-free medium (SFM)-101 (Nissui Pharmaceutical, Tokyo, Japan); fetal calf serum (FCS; Boehringer-Mannheim, Mannheim, Germany); and interleukin-1β (IL-1β; Genzyme, Cambridge, MA).

Preparation of Human Rheumatoid Synovial Cells. Synovial tissues were obtained from 10 Japanese patients (a-j) who met the revised American Rheumatism Association criteria for the classification of rheumatoid arthritis (Arnett et al., 1988) at the time of total knee replacement. Their serum concentrations of C-reactive protein (CRP) and erythrocyte sedimentation rates were 2.2 ± 0.3 mg/dL and 73.2 ± 34.4 mm/h, respectively. Steinbrocker stages of patients were stage III or stage IV. The rheumatoid synovial cells were prepared as described previously with modifications (Dayer et al., 1976; Brennan et al., 1989). The synovial tissues were digested for 2 h with 0.2% (w/v) collagenase type I and for 2 h with 0.125% (w/v) trypsin, and then suspended in Dulbecco’s modified Eagle’s medium containing 10% (v/v) FCS, 2 mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 25 ng/ml fungzone (Gibco, Grand Island, NY). The isolated cells, referred to as first-passage rheumatoid synovial cells in this report, were a heterogeneous mixture of all the infiltrating mononuclear cells and connective tissue cells found in synovial tissue. In some experiments, the cells were incubated at 37°C in 5% CO_2 until the adherent cells reached confluence, and nonadherent cells were removed. The adherent cells, referred to as the second-passage rheumatoid synovial cells in this report, were homogenous, presumably fibroblasts, compared with the first-passage cells.

Treatment of Rheumatoid Synovial Cells and Preparation of Culture Media. To estimate the production of PGE_2 and the metabolism of aceclofenac or 4'-hydroxyaceclofenac by first-passage rheumatoid synovial cells, the cells were suspended at 10^6 cells/ml in SFM-101 medium supplemented with 1% (v/v) FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin, and then various concentrations of drugs were added. After various periods of culture at 37°C in 5% CO_2, the culture media were harvested and stored at −80°C until use.

Detection of Microsomal COX Activity in Rheumatoid Synovial Cells. When the second-passage rheumatoid synovial cells reached confluency, 1 ng/ml of IL-1β was added. After 16 h in culture, the cells were suspended at 10^6 cells/ml in 20 mM potassium phosphate buffer (pH 7.4), and lysed by sonication. Microsomes were pelleted from the lysate by centrifugation at 105,000g for 1 h. The microsomal preparation was used for the assay of COX activity.

The COX activity was measured as described previously with modifications (Mitchell et al., 1994). Fifteen micrograms of microsomes was added to 200 µl of 50 mM Tris-HCl buffer (pH 8.0) containing glutathione (5 mM), epinephrine (5 mM), and hematin (1 µM) as cofactors. The mixture was preincubated with various concentrations of drugs for 10 min at 37°C, and then 6.6 µM arachidonic acid was added. After incubation at 37°C for 10 min, the reaction was stopped by adding 50 µl of 0.2 N HCl, and then 50 µl of 0.2 N NaOH was added to each sample. The preparation was centrifuged at 12,500g for 5 min, and the supernatant was harvested and stored at −80°C until the measurement of PGE_2 content.

Western Blotting for COX-1 and -2. Confluent second-passage rheumatoid synovial cells were lysed in solubilization buffer (10 mM Tris-HCl, pH 7.4, 1% (w/v) NP-40, 0.1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 0.15 M NaCl, 1 mM EDTA, and 10 µg/ml aprotinin), and then centrifuged at 12,500g for 30 min to remove the cell debris. The supernatant was mixed with a final concentration of 3.3% (w/v) trichloroacetic acid. The resultant precipitates were subjected to electrophoresis on a 10% (w/v) acrylamide slab gel under reducing conditions (Laemmli, 1970). The separated proteins in the gel were electrotransferred to an Immobilon polyvinylidene difluoride transfer membrane (Millipore, Bedford, MA). The membrane was reacted with rabbit anti-COX-1 or anti-human COX-2 antisera that was then complexed with alkaline phosphatase-conjugated anti-rabbit IgG antibody. Immunoreactive COX-1 or -2 was visualized indirectly using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium as substrates.

Detection of Aceclofenac and Its Metabolites by HPLC. The 400-µl samples were placed in vial tubes on the autosampler of an automated solid-phase extraction system, PROSPECT (Spark Holland, Emmen, the Netherlands) (Nielen et al., 1987), which was linked to a HPLC system. A cartridge containing C18 bonded phase (Varian SPP, Harbor City, CA) filled with Spark Holland was used for solid-phase extraction. The HPLC analysis was performed with an LC-Module 1 (Waters, Milford, MA) with a J’sphere ODS-M80 4.6 × 150 mm column (YMC, Kyoto, Japan). The mobile phase, 0.05% (w/v) trifluoroacetic acid/acetonitrile (59:41), was delivered at 1.5 ml/min. p-Hydroxybenzoic acid isobutyl ester (Tokyo Chemical Industry Co., Tokyo, Japan) was used as the internal standard. The eluate was monitored by ultraviolet absorption at 276 nm, and then aceclofenac and its metabolites were identified on the basis of their retention times.

Enzyme Immunoassay for PGE_2 and Determination of Protein. The amount of PGE_2 and the protein content were measured using a commercial enzyme immunoassay kit (Cayman) and BCA protein assay reagent (Pierce, Rockford, IL), respectively, according to the manufacturer’s protocol. The limit of the detection for PGE_2 is 7.8 pg/ml in the PGE_2 enzyme immunoassay kit.

Statistical Analysis. A least-squares linear regression analysis was used for calculation of the correlation coefficient. P values less than 0.05 were considered significant.

Results

Effects of Aceclofenac and Its Metabolites on PGE_2 Production by Rheumatoid Synovial Cells. The major metabolite of aceclofenac is 4'-hydroxyaceclofenac, and the minor metabolites are diclofenac and 4'-hydroxydiclofenac in human blood (Yanagawa et al., 1993; Bort et al., 1996b). We investigated the effects of these compounds on PGE_2 production by first-passage synovial cells freshly prepared from 10 patients with rheumatoid arthritis (Fig. 1 and Table
The first-passage synovial cells from all patients spontaneously produced more PGE$_2$ (201.2 ± 98.2 ng/10$^6$ cells/24 h; n = 10) than did the second-passage cells (4.0 ± 2.6 ng/10$^6$ cells/24 h; n = 5) without an exogenous stimulus such as IL-1β. Both aceclofenac and 4'-hydroxyaceclofenac suppressed the PGE$_2$ production by the first-passage synovial cells of all patients, with IC$_{50}$ values of 21.0 ± 13.0 nM (n = 10) and 304.6 ± 238.8 nM (n = 9), respectively. However, their suppressive potency against the PGE$_2$ production differed markedly among the cells from different patients. For example, the IC$_{50}$ values of aceclofenac and 4'-hydroxyaceclofenac in patient b were about 25-fold lower than those in patient c. Diclofenac and 4'-hydroxydiclofenac also suppressed PGE$_2$ production, with IC$_{50}$ values of 1.3 ± 0.6 nM (n = 10) and 16.9 ± 8.5 nM (n = 9), respectively. The differences in the IC$_{50}$ values of diclofenac and 4'-hydroxydiclofenac against the PGE$_2$ production among the patients were smaller than those of aceclofenac and 4'-hydroxyaceclofenac.

Indomethacin, used as a control, reduced the PGE$_2$ production.

**Effects of Aceclofenac and Its Metabolites on Microsomal COX Activity.** We investigated the effects of aceclofenac and its metabolites on human COX activity in the microsomes of IL-1β-treated second-passage rheumatoid synovial cells. As shown in Fig. 2, aceclofenac and 4'-hydroxyaceclofenac had no inhibitory effects on the human COX activity. In contrast, diclofenac and 4'-hydroxydiclofenac inhibited the human COX activity (IC$_{50}$: 0.032 and 0.54 μM, respectively).

**Effects of Aceclofenac and 4'-Hydroxyaceclofenac on COX-1 and -2 Protein Levels in Rheumatoid Synovial Cells.** We examined the effects of aceclofenac and 4'-hydroxyaceclofenac on the COX-1 and -2 protein levels in IL-1β-stimulated second-passage rheumatoid synovial cells by Western blotting (Fig. 3). In the untreated rheumatoid synovial cells, only COX-1 protein was detected. The treatment of the cells with IL-1β resulted in a marked enhancement of the expression of the COX-2 protein. In contrast, the COX-1 protein level was not affected. Both aceclofenac and 4'-hydroxyaceclofenac at concentrations sufficient for PGE$_2$ suppression had no effects on the COX-1 and -2 protein levels after treatment with IL-1β. Dexamethasone, used as an active control, suppressed the expression of COX-2 protein and PGE$_2$ production strongly without suppressing the COX-1 protein.

**Hydrolysis of Aceclofenac and 4'-Hydroxyaceclofenac by Rheumatoid Synovial Cells.** We analyzed the metabolites of aceclofenac and 4'-hydroxyaceclofenac produced by first-passage rheumatoid synovial cells. These drugs were added to the cells, followed by incubation for 24 h. The culture medium was then assayed by HPLC. As shown in Fig. 4B and D, aceclofenac and 4'-hydroxyaceclofenac were hydrolyzed to diclofenac and 4'-hydroxydiclofenac, respectively, by the rheumatoid synovial cells, whereas other metabolites were not detected by this system. In addition, the spontaneous degradation of aceclofenac and 4'-hydroxyaceclofenac to diclofenac and 4'-hydroxydiclofenac, respectively, in the medium was examined (Fig. 4, A and C). No significant conversion of aceclofenac and 4'-hydroxyaceclofenac was observed in those samples.

In addition, we investigated the hydrolysis of aceclofenac and its metabolites on microsomal COX activity from rheumatoid synovial cells. Microsomes from IL-1β-treated second-passage rheumatoid synovial cells were added to 200 μl of 50 mM Tris-HCl buffer (pH 8.0) containing glutathione (5 mM), epinephrine (5 mM), and hematin (1 μM) as cofactors. Mixture was preincubated with aceclofenac (●), 4'-hydroxyaceclofenac (○), diclofenac (■) or 4'-hydroxydiclofenac (□) for 10 min at 37°C, and then 6.6 μM arachidonic acid was added. After incubation at 37°C for 10 min, PGE$_2$ content was measured by enzyme immunoassay. Data are means ± S.D. of triplicate samples. Amount of PGE$_2$ formed in control sample was 1.60 ± 0.12 ng/ml. Results are representative of two different experiments.

**TABLE 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Aceclofenac</th>
<th>4'-Hydroxyaceclofenac</th>
<th>Diclofenac</th>
<th>4'-Hydroxydiclofenac</th>
<th>Indomethacin</th>
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<tr>
<td></td>
<td>IC$_{50}$ (nM)</td>
<td></td>
<td>IC$_{50}$ (nM)</td>
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<td>4.2</td>
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<tr>
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<td>0.3</td>
<td>4.0</td>
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<tr>
<td>c</td>
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<td>614.1</td>
<td>1.5</td>
<td>22.3</td>
<td>4.9</td>
</tr>
<tr>
<td>d</td>
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<td>98.1</td>
<td>1.7</td>
<td>12.5</td>
<td>5.1</td>
</tr>
<tr>
<td>e</td>
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<td>746.9</td>
<td>1.7</td>
<td>23.8</td>
<td>2.8</td>
</tr>
<tr>
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<td>N.T.</td>
<td>1.2</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>g</td>
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<td>319.4</td>
<td>1.6</td>
<td>20.9</td>
<td>9.2</td>
</tr>
<tr>
<td>h</td>
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<td>324.3</td>
<td>1.3</td>
<td>23.0</td>
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</tr>
<tr>
<td>i</td>
<td>16.6</td>
<td>199.6</td>
<td>1.2</td>
<td>15.0</td>
<td>6.6</td>
</tr>
<tr>
<td>j</td>
<td>28.2</td>
<td>289.5</td>
<td>2.1</td>
<td>26.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Mean ± S.D.$^a$</td>
<td>21.0 ± 13.0</td>
<td>304.6 ± 238.8</td>
<td>1.3 ± 0.6</td>
<td>16.9 ± 8.5</td>
<td>7.5 ± 5.4</td>
</tr>
</tbody>
</table>

$^a$ Not tested.

$^b$ Data are means ± S.D. of 9 or 10 patients (a-j).
and 4'-hydroxyaceclofenac to diclofenac and 4'-hydroxydiclofenac, respectively, by each first-passage rheumatoid synovial cell preparation. Aceclofenac and 4'-hydroxyaceclofenac (10 \(\mu M\)) were hydrolyzed to diclofenac (conversion rate: 7.7\%, \(p = 0.48\)) or 4'-hydroxydiclofenac (conversion rate: 4.0\%, \(p = 0.09\)) in all cell preparations. The hydrolytic activity of the first-passage cells was somewhat strong, and varied among the cell preparations compared with that of the second-passage cells. The hydrolytic activity in the first- and second-passage cells was not affected by IL-1\(\beta\) (data not shown).

**Relationship between Hydrolysis of Aceclofenac or 4'-Hydroxyaceclofenac and Their Inhibitory Effects on PGE\(_2\) Production in Rheumatoid Synovial Cells.** We examined the time course variation of the hydrolysis of aceclofenac and its inhibitory effect on PGE\(_2\) production in first-passage rheumatoid synovial cells. Aceclofenac was time-dependently converted into diclofenac by rheumatoid synovial cells (Fig. 5A). The time dependence was also recognized in the inhibitory effect of a lower dose of aceclofenac cells. We did not find a correlation between the hydrolysis of aceclofenac (\(r = 0.25, p = 0.48\)) or 4'-hydroxyaceclofenac (\(r = 0.26, p = 0.50\)) and the PGE\(_2\) production.

We compared the hydrolytic activity of first-passage synovial cells with that of subculture cells at the second passage. The hydrolytic activity for the conversion of 10 \(\mu M\) aceclofenac into diclofenac in the first- and second-passage cells (\(n = 5\)) was 1.03 \(\pm 0.51\) and 0.67 \(\pm 0.11\) \(\mu M/10^6\) cells/24 h, respectively, and the hydrolytic activity for the conversion of 10 \(\mu M\) 4'-hydroxyaceclofenac into 4'-hydroxydiclofenac was 0.57 \(\pm 0.40\) and 0.09 \(\pm 0.04\) \(\mu M/10^6\) cells/24 h, respectively. The hydrolytic activity of the first-passage cells was somewhat strong, and varied among the cell preparations compared with that of the second-passage cells. The hydrolytic activity in the first- and second-passage cells was not affected by IL-1\(\beta\) (data not shown).
(0.1 μM) on PGE\textsubscript{2} production, although a higher dose of aceclofenac (10 μM) inhibited PGE\textsubscript{2} production immediately (Fig. 5B). We examined the relationship between the suppressive effects of aceclofenac on PGE\textsubscript{2} production and the conversion of aceclofenac into diclofenac in the first-passage rheumatoid synovial cells. The relative suppressive potency of aceclofenac to diclofenac (IC\textsubscript{50} of diclofenac/IC\textsubscript{50} of aceclofenac) against the PGE\textsubscript{2} production in each cell preparation was plotted against the rate of the conversion of aceclofenac (%) in the corresponding cell preparation. The results indicated that there was a linear correlation between the degree of conversion and the relative suppressive potency of aceclofenac to diclofenac against the PGE\textsubscript{2} production in rheumatoid synovial cells (Fig. 6A). The relative suppressive potency of 4'-hydroxyaceclofenac to 4'-hydroxydiclofenac against the PGE\textsubscript{2} production was also correlated with the degree of the conversion of 4'-hydroxyaceclofenac into 4'-hydroxydiclofenac in the rheumatoid synovial cells (Fig. 6B).

**Discussion**

The results of the present study confirm and support our previous observations showing that aceclofenac, which had no direct inhibitory effects on COX-1 and -2 activities, suppressed PGE\textsubscript{2} production following its conversion into the COX inhibitors diclofenac and 4'-hydroxydiclofenac in inflammatory cells such as PMNs (Yamazaki et al., 1997).

In this study, aceclofenac and 4'-hydroxyaceclofenac suppressed the PGE\textsubscript{2} production by the synovial cells from all patients. It has been reported that the induction of COX-2 but not COX-1 by IL-1β in rheumatoid synovial cells is associated with an increase in PGE\textsubscript{2} production (Hulkower et al., 1994; Kawai et al., 1998). We obtained the same result here using a Western blot analysis. Therefore, the majority of COX activity in the microsomes of the IL-1β-treated rheumatoid synovial cells was COX-2 activity. Aceclofenac and 4'-hydroxyaceclofenac had no inhibitory effect on the microsomal COX activity in the rheumatoid synovial cells. In contrast, diclofenac and 4'-hydroxydiclofenac inhibited the COX activity. These results are similar to previous results obtained using COX-2 from sheep placenta (Yamazaki et al., 1997).

Masferrer et al. (1994) reported that dexamethasone suppressed the PGE\textsubscript{2} production following the reduction of COX-2 mRNA and protein expression. In contrast, NSAIDs suppressed PGE\textsubscript{2} production via their inhibitory effects on COX-1 and/or COX-2 activities (Vane, 1971). However, some of these NSAIDs, such as salicylic acid, a weak COX-1 inhibitor, were reported to decrease the COX-2 protein expression (Tordjman et al., 1995). We thus attempted to identify the regulatory effects of aceclofenac and 4'-hydroxyaceclofenac on the COX-1 and -2 protein levels. These drugs were found to have no suppressive effects on the COX-1 and -2 protein levels in rheumatoid synovial cells, although they inhibited PGE\textsubscript{2} production completely.

We also examined the hydrolysis of aceclofenac and 4'-hydroxyaceclofenac in first-passage synovial cells freshly isolated from 10 patients with rheumatoid arthritis by analyzing the metabolites in the culture medium using HPLC. The HPLC method was easier and more convenient for analyzing the metabolism of aceclofenac and 4'-hydroxyaceclofenac in many samples at one time compared with the thin-layer chromatography method, which we used in a preliminary study (Yamazaki et al., 1997). The HPLC data indicated that aceclofenac and 4'-hydroxyaceclofenac were hydrolyzed to diclofenac and 4'-hydroxydiclofenac, respectively, by the first-passage rheumatoid synovial cells. The amounts of diclofenac and 4'-hydroxydiclofenac produced were enough to suppress the PGE\textsubscript{2} production, as indicated by the concentration-response studies. However, the hydrolytic activity was different among the cell preparations from different patients. As mentioned above, aceclofenac and 4'-hydroxyaceclofenac suppressed PGE\textsubscript{2} production by the first-passage rheumatoid synovial cells. However, their IC\textsubscript{50} values were also different among the cell preparations from different patients. Furthermore, the differences in their IC\textsubscript{50} values were larger than those of diclofenac and 4'-hydroxydiclofenac. Therefore, we examined the relationship between the relative suppressive potency of aceclofenac to diclofenac against the PGE\textsubscript{2} production and the rate of the conversion of aceclofenac into diclofenac in each cell preparation. The results indicated that there was a clear and rigid correlation between the degree of conversion and the relative suppressive potency against the PGE\textsubscript{2} production in rheumatoid synovial cells. In the same way, the rate of the conversion of 4'-hydroxyaceclofenac into 4'-hydroxydiclofenac was also correlated with the relative suppressive potency of 4'-hydroxy-

**Fig. 6.** Correlation between hydrolysis of aceclofenac or 4'-hydroxyaceclofenac and their suppressive effects on PGE\textsubscript{2} production by rheumatoid synovial cells. First-passage rheumatoid synovial cells (10\textsuperscript{6} cells/ml) from 10 different patients (a-j) were cultured in the presence of aceclofenac (A) or 4'-hydroxyaceclofenac (B). After 24 h in culture, amounts of diclofenac or 4'-hydroxydiclofenac and PGE\textsubscript{2} content in culture medium were measured by HPLC and an enzyme immunoassay, respectively. Results are those of 10 separate experiments. ACF, aceclofenac; DCF, diclofenac; 4'-OH ACF, 4'-OH aceclofenac; 4'-OH DCF, 4'-OH diclofenac.
aceclofenac to 4'-hydroxydiclofenac against the PGE$_2$ production in rheumatoid synovial cells. Thus, it was suggested that the suppressive effects of aceclofenac and 4'-hydroxyaceclofenac on PGE$_2$ production were facilitated by the hydrolytic activity in the rheumatoid synovial cells.

The hydrolytic activity for the conversion of aceclofenac or 4'-hydroxyaceclofenac into diclofenac or 4'-hydroxydiclofenac in the first-passage cells was somewhat strong, and varied among the cell preparations compared with that in the second-passage cells. In the first-passage rheumatoid synovial cells, T cells were the predominant cell type and macrophages/monocytes were also common (Brennan et al., 1989). In contrast, these cells were not detected in the second-passage cells by two-color immunofluorescence and flow cytometry (data not shown). The population of second-passage cells was homogenous, presumably fibroblasts, compared with the first-passage cells. These results suggested that the difference of the cellular composition likely affected the hydrolytic activity in the rheumatoid synovial cells. In addition, we previously reported that the hydrolytic activity in the first-passage rheumatoid synovial cells was about four times greater than that in human normal dermal fibroblasts (Yamazaki et al., 1997). This preferential metabolism of aceclofenac into active metabolite may account for its good tolerance in vivo.

In summary, the present results demonstrated that the suppressive effects of aceclofenac and its major metabolite in human blood, 4'-hydroxyaceclofenac, on PGE$_2$ production were caused not by the inhibition of COX expression and COX activity but rather by their hydrolysis to the active metabolites diclofenac and 4'-hydroxydiclofenac in rheumatoid synovial cells. The hydrolytic activity was strongly correlated with the suppressive potency of aceclofenac and 4'-hydroxyaceclofenac against the PGE$_2$ production. These results suggest that aceclofenac is a new type of NSAID whose suppressive effect on the PGE$_2$ production is facilitated by hydrolytic activity at the site of inflammation, i.e., the rheumatoid synovium.

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References


Send reprint requests to: Ryuta Yamazaki, Yakult Central Institute for Microbiological Research, 1798 Yaho, Kunitachi-shi, Tokyo 186-8650, Japan.