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

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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 proportionally correlated with the hydrolytic activity in rheumatoid synovial cell preparations, we suggest that the suppressive effects of aceclofenac and 4'-hydroxy aceclofenac 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; Ward et al., 1995; Díaz et al., 1996). Aceclofenac also reduced PGE₂ levels in the synovial fluid of patients with acute knee pain (Cecchetin et al., 1988) and suppressed PGE₂ production by blood polymorphonuclear leukocytes (PMNs) or mononuclear cells from patients with severe osteoarthritis after its administration, similar to other general NSAIDs (González et al., 1994). Based on the structure of aceclofenac, and from the data about the rapid hydrolysis of aceclofenac to diclofenac in rats (Bort et al., 1996a), it was suggested that aceclofenac may be a prodrug of diclofenac. However, the major metabolite of aceclofenac in humans is 4'-hydroxyaceclofenac, whereas the level of diclofenac was shown to be low in human blood after aceclofenac treatment (Yanagawa et al., 1993; Bort et al., 1996b).

Our recent preliminary study (Yamazaki et al., 1997) demonstrated that aceclofenac and 4'-hydroxyaceclofenac had no inhibitory effect on COX activity. However, they suppressed PGE₂ production following their conversion into COX inhibitors, diclofenac and 4'-hydroxydiclofenac, in some inflammatory-related cells such as PMNs, monocytes, and rheumatoid synovial cells. In the present study, we further investigated the intracellular mechanisms of the action of aceclofenac 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 antiserum (Oxford Biomedical Research, Oxford, UK); rabbit anti-human COX-2 antiserum and the PGE\textsubscript{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 (II-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 ± 3.1 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 fungizone (Gibco, Grand Island, NY). The isolated cells, referred to as first-passage rheumatoid synovial cells, were cultured in the presence of drugs for 10 h, at 37°C in 5% CO\textsubscript{2} until the adherent cells reached confluence, and nonadherent cells were removed. The adherent cells, referred to as the second-passage rheumatoid synovial cells, 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\textsuperscript{5} cells/ml in 20 mM potassium phosphate buffer (pH 7.4), and lysed by sonication. Microsomes were pelleted from the lysate by centrifugation at 105,000 g for 1 h. 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\textsubscript{2} and the metabolites of acetylsalicylic acid or 4'-hydroxyacetylsalicylic acid by first-passage rheumatoid synovial cells, the cells were suspended at 10\textsuperscript{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\textsubscript{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\textsuperscript{5} cells/ml in 20 mM potassium phosphate buffer (pH 7.4), and lysed by sonication. Microsomes were pelleted from the lysate by centrifugation at 105,000 g 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\textsubscript{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 transferred to an Immobilon polyvinylidene difluoride transfer membrane (Millipore, Bedford, MA). The membrane was reacted with rabbit anti-COX-1 or anti-human COX-2 antiserum, which 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 by 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\textsubscript{2} and Determination of Protein. The amount of PGE\textsubscript{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 detection for PGE\textsubscript{2} was 7.8 pg/ml in the PGE\textsubscript{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\textsubscript{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\textsubscript{2} production by first-passage synovial cells freshly prepared from 10 patients with rheumatoid arthritis (Fig. 1 and Table 1).
1). 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-1b. 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'-oxyhydroxydiclofenac 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.

**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>a</td>
<td>4.1</td>
<td>121.6</td>
<td>0.2</td>
<td>4.2</td>
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<tr>
<td>b</td>
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<td>27.7</td>
<td>0.3</td>
<td>4.0</td>
<td>2.7</td>
</tr>
<tr>
<td>c</td>
<td>47.5</td>
<td>614.1</td>
<td>1.5</td>
<td>22.3</td>
<td>4.9</td>
</tr>
<tr>
<td>d</td>
<td>16.2</td>
<td>98.1</td>
<td>1.7</td>
<td>12.5</td>
<td>5.1</td>
</tr>
<tr>
<td>e</td>
<td>22.5</td>
<td>746.9</td>
<td>1.7</td>
<td>23.8</td>
<td>2.8</td>
</tr>
<tr>
<td>f</td>
<td>20.5</td>
<td>N.T.$^a$</td>
<td>1.2</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>g</td>
<td>29.4</td>
<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>
<td>15.2</td>
</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>
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<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).

**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. 4A and C). No significant conversion of aceclofenac and 4'-hydroxyaceclofenac was observed in those samples.

In addition, we investigated the hydrolysis of aceclofenac...
and 4'-hydroxyaceclofenac to diclofenac and 4'-hydroxydiclofenac, respectively, by each first-passage rheumatoid synovial cell preparation. Aceclofenac and 4'-hydroxyaceclofenac (10 μM) were hydrolyzed to diclofenac (conversion rate: 7.7 ± 4.0% and 4'-hydroxydiclofenac (conversion rate: 7.4%; n = 10) and 4'-hydroxydiclofenac (conversion rate: 6.54 ± 0.45%, n = 9), respectively, in all cell preparations after 24 h in culture. However, the degrees of the conversion of aceclofenac (range: 6.4–31.7%) and 4'-hydroxyaceclofenac (range: 3.2–14.1%) were different among the different cell preparations.

We examined the relationship between the hydrolysis of aceclofenac or 4'-hydroxyaceclofenac by the first-passage synovial cells and the CRP of the 10 patients. We did not find a correlation between the hydrolysis of aceclofenac (r = 0.25, p = 0.49) or 4'-hydroxyaceclofenac (r = 0.63, p = 0.07) and CRP. We also examined the relationship between the hydrolytic activity and the PGE₂ production by the first-passage 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 PGE₂ 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 μM aceclofenac into diclofenac in the first- and second-passage cells (n = 5) was 1.03 ± 0.51 and 0.67 ± 0.11 μM/10⁶ cells/24 h, respectively, and the hydrolytic activity for the conversion of 10 μM 4'-hydroxyaceclofenac into 4'-hydroxydiclofenac was 0.57 ± 0.40 and 0.09 ± 0.04 μM/10⁶ 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-1β (data not shown).

Relationship between Hydrolysis of Aceclofenac or 4'-Hydroxyaceclofenac and Their Inhibitory Effects on PGE₂ Production in Rheumatoid Synovial Cells. We examined the time course variation of the hydrolysis of aceclofenac and its inhibitory effect on PGE₂ 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.


(0.1 μM) on PGE₂ production, although a higher dose of aceclofenac (10 μM) inhibited PGE₂ production immediately (Fig. 5B). We examined the relationship between the suppressive effects of aceclofenac on PGE₂ production and the conversion of aceclofenac into diclofenac in the first-passage rheumatoid synovial cells. The relative suppressive potency of aceclofenac to diclofenac (IC₅₀ of diclofenac/IC₅₀ of aceclofenac) against the PGE₂ 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 PGE₂ production in rheumatoid synovial cells (Fig. 6A). The relative suppressive potency of 4'-hydroxyaceclofenac to 4'-hydroxydiclofenac against the PGE₂ 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 PGE₂ 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 PGE₂ 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 PGE₂ 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 PGE₂ production following the reduction of COX-2 mRNA and protein expression. In contrast, NSAIDs suppressed PGE₂ 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 PGE₂ 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 PGE₂ 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 PGE₂ production by the first-passage rheumatoid synovial cells. However, their IC₅₀ values were also different among the cell preparations from different patients. Furthermore, the differences in their IC₅₀ 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 PGE₂ production and the rate of 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 PGE₂ production in rheumatoid synovial cells. In the same way, the rate of conversion of 4'-hydroxyaceclofenac into 4'-hydroxydiclofenac was also correlated with the relative suppressive potency of 4'-hydroxy-
aceclofenac to 4′-hydroxydiclofenac against the PGE2 production in rheumatoid synovial cells. Thus, it was suggested that the suppressive effects of aceclofenac and 4′-hydroxyaceclofenac on PGE2 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 metabolites may account for its good tolerability, although the mechanism of the regulation of hydrolytic activity in rheumatoid synovial cells remains unclear.

In summary, the present results demonstrated that the suppressive effects of aceclofenac and its major metabolite in the human blood, 4′-hydroxyaceclofenac, on PGE2 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 PGE2 production. These results suggest that aceclofenac is a new type of NSAID whose suppressive effect on the PGE2 production is facilitated by hydrolytic activity at the site of inflammation, i.e., the rheumatoid synovium.

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References


