DICLOFENAC-INDUCED INACTIVATION OF CYP3A4 AND ITS STIMULATION BY QUINIDINE

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ABSTRACT:

Incubation of human liver microsomes with diclofenac in the presence of NADPH resulted in a decrease in testosterone 6β-hydroxylation activity. The decrease in the activity followed time- and concentration-dependent kinetics, required oxidative metabolism, and was resistant to reduced glutathione, suggesting that diclofenac causes a mechanism-based inactivation of cytochrome P450 (P450) 3A4 (CYP3A4). The inactivation was reproduced by using microsomes from B-lymphoblastoid cell lines expressing CYP3A4 instead of human liver microsomes. No other monooxygenase activities measured as indexes of P450 enzymes; CYP2C8, CYP2C9, or CYP2C19 was inactivated by the same incubation procedure. Quinidine, a stimulant of CYP3A4-mediated diclofenac 5-hydroxylation, did not affect the inactivation of CYP3A4 assessed by testosterone 6β-hydroxylation activity but accelerated the inactivation assessed by diazepam 3-hydroxylation activity. These results support the idea that diclofenac 5-hydroxylation is involved in the inactivation of CYP3A4 and described for the first time a stimulation of mechanism-based inactivation attributable to CYP3A4 heterotropic cooperativity. Preincubation of human liver microsomes with 5-hydroxydiclofenac instead of diclofenac did not cause the inactivation of CYP3A4, suggesting that 5-hydroxydiclofenac is not a precursor of a postulated reactive metabolite that inactivates CYP3A4, and thus 5-hydroxylation step is critical to inactivation of CYP3A4.

Mechanism-based inactivation of cytochrome P450 (P450) is a serious clinical problem because it causes undesirable drug accumulation and drug-drug interaction. In basic researches, mechanism-based inactivators are valuable for identifying a P450 isoform responsible for a specific monooxygenase reaction because of their higher selectivities than those of competitive inhibitors. The property of these compounds to bind covalently to specific amino acid residues of specific P450 also allowed us to identify the active site of the P450 enzyme (Halpert, 1995; Kent et al., 2001).

We recently found that diclofenac, a nonsteroidal anti-inflammatory drug, was a potent mechanism-based inactivator of CYP2C11, a predominant P450 isoform in male rat liver (Masubuchi et al., 2001). CYP2C11 was shown to be responsible for major diclofenac metabolisms, 4′-hydroxylation and 5-hydroxylation (Fig. 1), in rat liver microsomes (Masubuchi et al., 2001), whereas distinct P450 isoforms, CYP2C9 and CYP3A4, were involved in these pathways, respectively, in human liver microsomes (Leemann et al., 1993; Shen et al., 1999; Tang et al., 1999b). Because CYP2C9 was not inactivated during diclofenac metabolism (Masubuchi et al., 2001), it was suggested that the inactivation of CYP2C11 was independent of 4′-hydroxylation pathway, supposing alternatively that 5-hydroxylation is involved in the inactivation of CYP2C11. Thus in humans, it is possible that CYP3A4, a major isoform responsible for diclofenac 5-hydroxylation, is susceptible to inactivation by diclofenac metabolism. It has been demonstrated that benzoquinone imine, a further metabolite of 5-hydroxydiclofenac, is chemically reactive and binds covalently to cellular protein (Shen et al., 1999; Tang et al., 1999b). Although implication of 5-hydroxydiclofenac for diclofenac-induced liver toxicity has been suggested, the effect on CYP3A4 catalytic ability has not been investigated. The present study focused on the effect of diclofenac metabolism on CYP3A4 in human liver microsomes and recombinant CYP3A4.

Materials and Methods

Chemicals. Diclofenac sodium and reduced glutathione (GSH) were purchased from the Wako Pure Chemicals (Osaka, Japan); testosterone, paclitaxel, and quinidine hydrochloride were from Sigma-Aldrich (St. Louis, MO); 6β-hydroxytestosterone was from Steraloids Inc. (Wilton, NH); 6α-hydroxycladitaxel was from BD Gentest (Woburn, MA); 4′-Hydroxyclofenac and 5-hydroxydiclofenac were gifts from Novartis Pharma AG (Basel, Switzerland); diazepam, 3-hydroxydiazepam (temazepam) and N-desmethyldiazepam (nor-diazepam) were gifts from Nippon Roche Co., Ltd. (Tokyo, Japan). Glucose 6-phosphate (G-6-P), glucose 6-phosphate dehydrogenase (G-6-PDH) and NADPH were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other chemicals and solvents used were of analytical grade.

Liver Microsomes and P450 Enzyme. Human liver microsomes (pooled fraction from 15 patients) were purchased from Tissue Transformation Technologies (Edison, NJ). Microsomal preparations from B-lymphoblastoid cell lines expressing CYP3A4 were purchased from BD Gentest.

Preincubation of Liver Microsomes with Diclofenac and Its Metabolites. Pooled human liver microsomes or microsomes from B-lymphoblastoid cells expressing CYP3A4 were preincubated with diclofenac in the presence of NADPH, to determine effects of the metabolic intermediates on microsomal...
monooxygenase activities. A 0.5-ml incubation mixture contained 0.25 mg of human liver microsomal protein (or 0.125 mg of microsomes from B-lymphoblastoid cells), 10 mM G-6-P, 1 unit G-6-PDH, 10 mM MgCl₂, 0.1 mM EDTA and various concentrations of diclofenac in 0.15 M potassium phosphate buffer (pH 7.4). In some experiments, 4'-hydroxydiclofenac or 5-hydroxydiclofenac was employed instead of diclofenac. After temperature equilibration (37 °C), 5 min), preincubation of microsomes with diclofenac was started by adding NADPH (final 0.5 mM) and performed for various time periods up to 30 min. The subsequent incubation of the microsomes for the assay of enzymatic activities was started by the addition of a test substrate, testosterone, paclitaxel, or diazepam.

**Assay of Testosterone 6β-Hydroxylation and Paclitaxel 6α-Hydroxylation Activities.** Testosterone 6β-hydroxylation activity, an indicator of CYP3A4, of the preincubated microsomes was determined according to the HPLC method (Masubuchi et al., 1995). After the preincubation of the microsomes with diclofenac or its metabolite as described above, testosterone at the final concentration of 0.2 mM was added, and the incubation was performed for 2 min. Reaction was terminated by ethyl acetate, and then phenacetin was added to the mixture as an internal standard. 6β-Hydroxytestosterone was extracted into ethyl acetate, the organic layer was evaporated to dryness, and the residue was dissolved in 0.1 ml of a mobile phase for the HPLC, which consisted of acetonitrile, methanol, and water (2:4:3, by volume). The sample was applied to a reversed-phase column (Inertsil ODS; GL Sciences Ltd., Tokyo, Japan). The UV absorbance intensity of 6β-hydroxytestosterone was monitored at 254 nm. Paclitaxel 6α-hydroxylation activity, an indicator of CYP2C8, of the preincubated microsomes was determined according to the HPLC method (Desai et al., 1998) with modifications. The incubation was performed at the paclitaxel concentration of 50 μM for 5 min. Reaction was terminated by ethyl acetate, and then 2-acetamidophenol was added to the mixture as an internal standard. 6α-Hydroxypaclitaxel was extracted into ethyl acetate, the organic layer was evaporated to dryness, and the residue was dissolved in 0.1 ml of a mobile phase for the HPLC, which consisted of acetonitrile and water (65:35, by volume). The sample was applied to a reversed-phase column (Inertsil ODS). The UV absorbance intensity of 6α-hydroxypaclitaxel was monitored at 230 nm. The assays were performed under linear conditions of metabolite formation with regard to incubation time and protein concentration.

**Assay of Diazepam 3-Hydroxylation and N-Demethylation Activities.** CYP3A4 is known to be a major isozyme involved in diazepam 3-hydroxylation and high-Kₘ diazepam N-demethylation, whereas CYP2C19 is a low-Kₘ diazepam N-demethylese (Yasumori et al., 1993). In the present study, diazepam N-demethylation at the substrate concentration of 20 μM was assayed as a representative of CYP2C19, and diazepam 3-hydroxylation at the concentration of 200 μM was as a representative of CYP3A4. Diazepam N-demethylation and 3-hydroxylation activities were determined according to the HPLC methods (Fujita et al., 1990) with modifications. After the preincubation of the microsomes with diclofenac or its metabolite as described above, the incubation was started by adding diazepam and was performed for 2 min. Reaction was terminated by ethyl acetate, and then nitrazepam was added to the mixture as an internal standard. The diazepam metabolites were extracted into ethyl acetate, the organic layer was evaporated to dryness, and the residue was dissolved in 0.1 ml of a mobile phase for the HPLC, which consisted of acetonitrile, methanol, and water (5:70:35, by volume). The sample was applied to a reversed-phase column (Inertsil ODS). The UV absorbance intensity of temazepam and nordiazepam was monitored at 230 nm.

**Assay of Diclofenac 4'- and 5-Hydroxylation Activities.** Diclofenac 4'- and 5-hydroxylation activities were assayed according to the HPLC method (Masubuchi et al., 2001) with modifications. A 0.5-ml incubation mixture contained 0.25 mg of liver microsomes, 10 mM G-6-P, 1 unit G-6-PDH, 5 mM MgCl₂, and 500 μM diclofenac in 0.15 M potassium phosphate buffer (pH 7.4). After temperature equilibration (37 °C, 5 min), the reaction was started by adding NADPH (final 0.5 mM), and the incubation was performed for 30 min. Reaction was terminated by 1 M sodium phosphate buffer (pH 5.0), and then flurbiprofen was added to the mixture as an internal standard. The metabolites of diclofenac were extracted into diethyl ether, the organic layer was evaporated to dryness, and the residue was dissolved in 0.1 ml of a mobile phase for the HPLC, which consisted of 100 mM potassium phosphate buffer (pH 7.25) and acetonitrile (7:3, by volume). The sample was applied to a reversed-phase column (Inertsil ODS-3; GL Sciences Ltd.). The UV absorbance intensity of diclofenac metabolites was monitored at 282 nm.

**Data Analysis.** Pseudofirst order kinetic constants for the enzyme inactivation (k) were calculated from the initial slopes of the linear regression lines of the semilogarithmic plots of the remaining enzyme activity against the preincubation time. The reciprocal of k thus obtained was plotted against the reciprocal of the diclofenac concentration, and then a concentration required for a half-maximal inactivation (Kₐ) for the inactivation and a maximal inactivation rate constant (kₐmax) were determined from the intercepts on the absissa and the ordinate, respectively.

**Results**

**Mechanism-Based Inactivation of CYP3A4 by Diclofenac.** Addition of diclofenac inhibited human liver microsomal testosterone 6β-hydroxylation activity (plots on y-axis in Fig. 2) and the IC₅₀ was calculated to be higher than 1.0 mM. Preincubation of the microsomes with diclofenac in the presence of NADPH intensified the inhibitory potency (Fig. 2). The preincubation under the same condition but in the absence of NADPH did not lead to the time-dependent loss of testosterone 6β-hydroxylation activity (data not shown). These results indicated that CYP3A4 was inactivated during the oxidative metabolism of diclofenac. Decreases in the activity with respect to the preincubation time followed first order rate kinetics. The first order kinetic constants for the enzyme inactivation (k) were obtained from initial slopes for semilogarithmic plots at various diclofenac concentrations (Fig. 2B). The double-reciprocal plots of diclofenac concentrations versus the k values provided the Kᵢ and kₐmax values of 1.64 ± 0.31 mM and 0.246 ± 0.082 min⁻¹, respectively. GSH at the concentration of 5 mM did not affect the loss of testosterone 6β-hydroxylation activities during the preincubation of the microsomes with diclofenac (30 min, 500 μM) in the presence of NADPH (without GSH, 56.7 ± 2.5; with GSH, 57.7 ± 1.6; % of the control activity obtained by the preincubation without diclofenac). These results suggested that diclofenac caused the mechanism-based inactivation of CYP3A4. The inactivation of CYP3A4 was reproduced by the experiments with recombinant CYP3A4; namely preincubation of microsomes from B-lymphoblastoid cell lines expressing CYP3A4 instead of liver microsomes with diclofenac in the presence of NADPH also resulted in time-dependent loss of testosterone 6β-hydroxylation activity (Fig. 3). We have already shown that diclofenac metabolism...
does not cause inactivation of CYP2C9 (Masubuchi et al., 2001), a major isozyme involved in diclofenac 4'-hydroxylation (Leemann et al., 1993). In the present study, effects on other CYP2C forms, which have been suggested to be involved in diclofenac metabolism, CYP2C8 and CYP2C19 (Bort et al., 1999), were assessed by paclitaxel 6α-hydroxylation activity and diazepam N-demethylation activity at a lower substrate concentration, respectively, and no clear time-dependent decrease in the activity was observed (data not shown).

Stimulation of Diclofenac-Induced Inactivation of CYP3A4 by Quinidine. Because CYP3A4, a major component of diclofenac 5-hydroxylation (Shen et al., 1999; Tang et al., 1999b), was selectively inactivated, it is reasonable to postulate that the 5-hydroxylation pathway of diclofenac is responsible for the inactivation of CYP3A4. Because recent reports (Tang et al., 1999a; Ngui et al., 2000) showed that diclofenac 5-hydroxylation was stimulated by quinidine, the effect of the stimulation on the inactivation of CYP3A4 was investigated. As previously shown (Ngui et al., 2000), a marked stimulation by the addition of quinidine at the concentration of 20 μM was observed in diclofenac 5-hydroxylation (control, 0.038 ± 0.010; quinidine 0.141 ± 0.029, nmol/min/mg of protein), but not in diclofenac 4'-hydroxylation (control, 0.509 ± 0.083; quinidine 0.453 ± 0.081, nmol/min/mg of protein) in human liver microsomes. Quinidine activated neither testosterone 6β-hydroxylation activity as reported previously (Ludwig et al., 1999) nor diazepam 3-hydroxylation activity. Addition of quinidine did not affect the time-dependent decrease in testosterone 6β-hydroxylation activity in human liver microsomes (Fig. 4A). But interestingly, quinidine accelerated the inactivation of CYP3A4 when diazepam was used instead of testosterone as a test substrate for CYP3A4 activity (Fig. 4B). As a result, time-dependent decrease in diazepam 3-hydroxylation activity was observed even at the diclofenac concentration that did not originally cause inactivation without quinidine (Fig. 5). The quinidine-induced stimulation of the CYP3A4 inactivation by diclofenac as assessed by diazepam 3-hydroxylation activity was also observed when recombinant CYP3A4 was used instead of human liver microsomes (Fig. 6).

Lack of Involvement of Primary Metabolites of Diclofenac and Their Further Metabolites in Diclofenac-Induced Inactivation of CYP3A4. We finally investigated whether diclofenac 5-hydroxylation or further metabolism of 5-hydroxydiclofenac was relevant to the inactivation of CYP3A4, the latter of which generates the postulated reactive benzoquinone imine (Shen et al., 1999; Tang et al., 1999b). Preincubation of human liver microsomes in the absence or presence of NADPH with 5-hydroxydiclofenac, instead of diclofenac, at the concentration of 100 μM, 250 μM, 500 μM, or 1000 μM, followed by assay of testosterone 6β-hydroxylation activity during preincubation of microsomes with diclofenac. In the presence of NADPH, followed by assay of testosterone 6β-hydroxylation activities during preincubation of microsomes with diclofenac.

FIG. 2. Time- and concentration-dependent decrease in testosterone 6β-hydroxylation activities during preincubation of microsomes with diclofenac.

Microsomes from pooled human livers were preincubated without (○) or with diclofenac (100 μM, ●, 250 μM, ■, 500 μM, △; 1000 μM, ▲) in the presence of NADPH, followed by assay of testosterone 6β-hydroxylation activities. Figure 2B is the reexamination of Fig. 2A but at the time period for preincubation to 5 min to determine initial slopes for the enzyme inactivation. Results are means ± S.E. (n = 3).

FIG. 3. Time-dependent decrease in testosterone 6β-hydroxylation activity of recombinant CYP3A4 during diclofenac metabolism.

Microsomes from B-lymphoblastoid cell lines expressing CYP3A4 were preincubated without (○) or with diclofenac (500 μM) (●) in the presence of NADPH, followed by assay of testosterone 6β-hydroxylation activity. Results are means ± S.E. (n = 3).
concentration of 1 μM that was actually generated by the diclofenac metabolism under the present conditions (diclofenac 500 μM; incubation, 30 min) did not cause a decrease in testosterone 6β-hydroxylation (A) or diazepam 3-hydroxylation (B) activities. Results are represented as percent of the initial activity obtained without the preincubation and are means ± S.E. (n = 3). * and **, significantly different from control obtained without quinidine (p < 0.05, p < 0.01, respectively) calculated by the Student’s t test.

Fig. 5. Time-dependent decrease in diazepam 3-hydroxylation activities during preincubation of microsomes with diclofenac in the presence of quinidine.

Microsomes from pooled human livers were preincubated with diclofenac (100 μM) and NADPH in the absence (●) or presence (▲) of quinidine (20 μM), followed by assay of diazepam 3-hydroxylation activities. Dotted lines represent controls preincubated without diclofenac in the absence (□) or presence (△) of quinidine. Results are means ± S.E. (n = 3).

Fig. 6. Decrease in diazepam 3-hydroxylation activity of recombinant CYP3A4 during diclofenac metabolism in the presence of quinidine.

Microsomes from B-lymphoblastoid cell lines expressing CYP3A4 were preincubated without or with diclofenac (100 μM) in the presence of NADPH, and in the absence (open column) or presence of quinidine (20 μM, hatched column) for 30 min, followed by assay of diazepam 3-hydroxylation activities. Results are represented as percent of the initial activity obtained without the preincubation, and are means ± S.E. (n = 3). *, significantly different from the relative activity obtained without quinidine (p < 0.05) calculated by the Student’s t test.

Discussion

The present study demonstrated that CYP3A4 was inactivated during metabolism of diclofenac. The inactivation 1) required...
by assay of testosterone 6'-hydroxylation activities.

Microsomes were not preincubated (open column) or preincubated for 30 min (hatched column) with diclofenac (D, 500 µM), 4'-hydroxydiclofenac (4'-OH-D, 5 µM), or 5-hydroxydiclofenac (5-OH-D, 1 µM) in the presence of NADPH, followed by assay of testosterone 6β-hydroxylation (A) and diazepam 3-hydroxylation (B) activities. Results are represented as percent of the control activity obtained without diclofenac or the metabolite and are means ± S.E. (n = 3). **, significantly different from the corresponding open column (p < 0.01) calculated by the Student's t test.

NADPH (oxidative metabolism), 2) followed pseudo-first order kinetics, 3) was saturable against increasing diclofenac concentration, 4) was protective against GSH, and 5) revealed selectivity toward specific P450 isoform(s). These characteristics of diclofenac meet the criteria for a mechanism-based inactivator, and it demonstrated that diclofenac was a novel CYP3A4 inactivator. We should mention here that the inactivation of CYP3A4 might not be important in the clinical setting, because the diclofenac concentrations to cause the inactivation ($K_i > 1$ mM) were much higher than blood concentrations of this drug in the clinical use (a low micromolar range). CYP3A4 and CYP2C9 are major P450 isozymes responsible for diclofenac 5-hydroxylation and 4'-hydroxylation, respectively (Leemann et al., 1993; Shen et al., 1999; Tang et al., 1999b), and only CYP3A4 was inactivated, implying involvement of diclofenac 5-hydroxylation in the generation of a reactive metabolite(s) to bind to CYP3A4. The inactivation required relatively high diclofenac concentrations, corresponding to the fact that diclofenac 5-hydroxylation is a high-$K_i$ reaction as compared with 4'-hydroxylation (Bort et al., 1999). Thus we further investigated the potential role of diclofenac 5-hydroxylation in the inactivation of CYP3A4.

Quinidine has been known as both a substrate of CYP3A4 and a potent inhibitor of CYP2D6 (Guengerich et al., 1986). Recently, it was reported that quinidine stimulated several CYP3A4-dependent monoxygenase activities including diclofenac 5-hydroxylation (Ludwig et al., 1999; Tang et al., 1999a; Ngui et al., 2000). Therefore, it was possible that quinidine potentiated the diclofenac-induced inactivation of CYP3A4 through the stimulation of diclofenac 5-hydroxylation. Contrary to the expectation, the inactivation of CYP3A4 during the diclofenac metabolism was not affected by the coincubation of quinidine when testosterone was originally used as a test substrate. However when diazepam was employed instead of testosterone, the inactivation was accelerated by the coincubation with quinidine. It was thus concluded that stimulation of 5-hydroxylation results in stimulation of CYP3A4 inactivation, whereas additional explanation is necessary for different results obtained from different test substrates.

To provide mechanistic explanation for non-Michaelis-Menten kinetics in some CYP3A4 reactions and stimulation of some CYP3A4 reactions by other compounds, a number of reports have proposed that the CYP3A4 substrate binding site has multiple domains (Shou et al., 1994, 1999; Ngui et al., 1997; Hosea et al., 2000; Atkins et al., 2001). In the multibinding site theory, an effector site is also assumed, and binding of a substrate or an effector to this site results in increase in catalytic activity for the specific substrate, corresponding to homotropic cooperativity or heterotropic cooperativity, respectively (Shou et al., 1994, 1999; Ngui et al., 1997; Hosea et al., 2000; Atkins et al., 2001). Recent studies suggested that steroids such as testosterone and benzodiazepines such as diazepam bind to other binding domains in the CYP3A4 binding pocket (Kenworthy et al., 1999, 2001) (termed tentatively “testosterone site” and “diazepam site”). Because diclofenac caused metabolism-dependent decreases in both testosterone 6β-hydroxylation and diazepam 3-hydroxylation activities, it is considered that diclofenac binds both to the testosterone and diazepam sites, is metabolized to reactive intermediates, and results in covalent binding at these sites. On the other hand, the stimulation by quinidine, which binds probably to the third site, is stimulatory only to metabolism of diclofenac that binds to diazepam site. Quinidine did not stimulate diazepam 3-hydroxylation, clearly demonstrating that the stimulation is substrate-dependent even though both of the substrates (diclofenac and diazepam) bind to the same domain. Recently, it was reported that midazolam inactivated CYP3A4-dependent triazolam 3-hydroxylation and testosterone 6β-hydroxylation, but to different extents (Schrag and Wienkers, 2001), providing novel experimental evidence for existence of the multiple binding sites in CYP3A4. The present study provides additional evidence for the multiple domain proposals by discriminating stimulatory effects of quinidine on diclofenac 5-hydroxylation leading to enhanced inactivation of CYP3A4.

We examined whether diclofenac 5-hydroxylation or further metabolism of 5-hydroxydiclofenac was relevant to the inactivation of CYP3A4, the latter of which generates the postulated reactive benzoquinone imine (Shen et al., 1999; Tang et al., 1999b). The study with the primary metabolites of diclofenac indicated that CYP3A4 was not inactivated during the metabolism of 5-hydroxydiclofenac or 4'
hydroxydiclofenac. Lack of involvement of further metabolites in diclofenac-induced mechanism-based inactivation was similar to the event in inactivation of CYP2C11 (Masubuchi et al., 2001). It has been demonstrated that benzoquinone imine, a further metabolite of 5-hydroxydiclofenac, is chemically reactive and binds covalently to cellular protein, suggesting the involvement of the toxicity of diclofenac (Shen et al., 1999; Tang et al., 1999b). It is reasonable to speculate that reactive intermediates relevant to mechanism-based inactivation of CYP3A4 are too reactive to diffuse from the catalytic site of CYP3A4 and to bind other targets, whereas benzoquinone imine is relatively stable and can access to proteins other than P450s. The typical example revealing correlation between the stability and the target of a reactive metabolite is acetaminophen and its analog. Acetaminophen is a well known hepatotoxic compound, which is converted to a reactive benzoquinone imine and binds to hepatocellular proteins as the initial step of the toxicity, whereas its regioisomer, 3′-hydroxyacetaminid, is converted to a similar but highly reactive benzoquinonone(s), resulting in inactivation of P450 but not hepatotoxicity (Halmes et al., 1998). It is concluded that an intermediate during diclofenac 5-hydroxylation and a further metabolite of 5-hydroxydiclofenac lead to different toxicological consequences. We have not currently identified a chemical structure of the reactive metabolite to bind to CYP3A4, whereas arene-oxide is one of the possible candidates to be generated during diclofenac 5-hydroxylation. Because diclofenac 4′-hydroxylation by CYP2C9 does not result in its inactivation despite a higher metabolic rate than 5-hydroxylation, the two aromatic hydroxylations seem to proceed via distinct steps.

In summary, it was found that diclofenac was a novel mechanism-based inactivator of CYP3A4. Quinidine, a stimulant of diclofenac 5-hydroxylation also stimulated inactivation of CYP3A4. 5-Hydroxydiclofenac was shown not to be a precursor of inactivating species; therefore, the 5-hydroxylation step is important in inactivation of CYP3A4.

References


