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Research Paper

Identification of Human UDP-Glucuronosyltransferase Responsible for the Glucuronidation of Niflumic Acid in Human Liver

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Purpose. To assess the uridine diphosphate (UDP)-glucuronosyltransferase (UGT) isozymes involved in the glucuronidation of niflumic acid in human liver.
Methods. The glucuronidation activity of niflumic acid was determined in liver microsomes and recombinant UGT isozymes by incubation of niflumic acid with UDP-glucuronic acid (UDPGA).
Results. Incubation of niflumic acid with liver microsomes and UDPGA produced one peak, which was identified as a glucuronide from mass spectrometric analysis. A study involving a panel of recombinant human UGT isozymes showed that glucuronidation activity was highest in UGT1A1 among the isozymes investigated. The glucuronidation in human liver microsomes (HLMs) followed Michaelis-Menten kinetics with a K_m value of 16 μM, which is similar to that found with recombinant UGT1A1. The glucuronidation activity of niflumic acid in microsomes from eight human livers significantly correlated with UGT1A1-catalyzed estradiol 3β-glucuronidation activity ($r = 0.78$, $p < 0.05$). β-Estradiol inhibited niflumic acid glucuronidation with an IC_{50} of 25 μM in HLMs, comparable to that for UGT1A1.
Conclusions. These findings indicate that UGT1A1 is the main isozyme involved in the glucuronidation of niflumic acid in the human liver.

KEY WORDS: glucuronidation; niflumic acid; NSAID; UGT1A1.

INTRODUCTION

Niflumic acid (Fig. 1) is an anti-inflammatory drug that is effective for the treatment of rheumatoid diseases (1). After oral administration of niflumic acid to humans, it is rapidly absorbed (2) and extensively metabolized, mainly into hydroxide and glucuronide (3). Our previous studies showed that niflumic acid inhibited human uridine diphosphate (UDP)-glucuronosyltransferase (UGT). Niflumic acid inhibited estradiol 3β-glucuronidation, a UGT1A1 probe reaction, in human liver microsomes (HLMs) with an IC_{50} value of 22.2 μM (4). It also inhibited the glucuronidation of 4-methylumbelliferone in recombinant human UGT1A9 (5). Niflumic acid inhibited glucuronidation of mycophenolic acid in HLMs with an IC_{50} value of 8 μM (6,7). It was reported that mycophenolic acid was glucuronidated mainly by UGT1A9 (8). Thus, niflumic acid is considered to have a broad human UGT isozyme inhibitory spectrum.

Although it has been reported that UGT1A3, 1A8, 1A9, 1A10, and 2B7 all glucuronidate niflumic acid (9), the investigation of the main isozymes responsible for the glucuronidation of niflumic acid in the human liver remains to be carried out. The identification of the enzymes responsible for drug metabolism is important for understanding the variations in drug concentration that can lead to variation in drug efficacy and/or toxicity. It also provides essential information about drug–drug interactions. As specific inhibitors for respective UGT isozyme have rarely been reported, it is difficult to identify the main UGT isozymes involved in the glucuronidation of drugs. The glucuronidation of β-estradiol at the 3-OH position, trifluoperazine, and propofol were reported to be probe reactions for UGT1A1, 1A4, and 1A9, respectively. Thus, correlation studies between niflumic acid glucuronidation and these probe reactions in HLMs, along with a comparison of the kinetics of glucuronidation between recombinant human UGT isozymes and liver microsomes, help us to investigate the UGT isozymes responsible.

In this study, the main UGT isozyme involved in the glucuronidation of niflumic acid was assessed by using HLMs and recombinant human UGT isozymes.

MATERIALS AND METHODS

Chemicals and Reagents

Niflumic acid, β-estradiol, and 4-methylumbelliferone (4-MU) were purchased from Sigma (St. Louis, MO, USA). Pooled HLMs, individual HLMs, and a panel of recombinant human UGT Supersomes™ (UGT1A1, 1A3, 1A4, 1A6, 1A8, 1A9, 1A10, 2B7, and 2B15) expressed in baculovirus-infected insect cells were purchased from BD Biosciences (Woburn,

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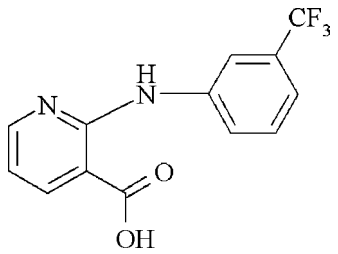

Fig. 1. Chemical structure of niflumic acid.

MA, USA). The glucuronidation activity in UGT Supersomes™ obtained from the manufacturer was as follows: 1000 pmol min^{-1} mg $protein^{-1}$ for estradiol 3β-glucuronidation (E3G) in UGT1A1, 150 pmol min^{-1} mg $protein^{-1}$ for E3G in UGT1A3; 660 pmol min^{-1} mg $protein^{-1}$ for 7-hydroxy-4-trifluoromethylcoumarin glucuronidation (7HTG) in UGT1A8; 7600, 240, and 1200 pmol min^{-1} mg $protein^{-1}$ for 7HTG in UGT1A9, UGT1A10, and UGT2B7, respectively. The glucuronidation activities in pooled HLMs for UGT1A1, 1A4, and 1A9 were 1100, 570, and 4800 pmol min^{-1} mg $protein^{-1}$, respectively. β-Glucuronidase was purchased from Sigma. All other chemicals were either of the highest grade or of high-performance liquid chromatography (HPLC) grade.

Glucuronidation of Niflumic Acid

Niflumic acid was incubated in reaction mixtures of 0.25 mL of 50 mM Tris–HCl buffer (pH 7.5) containing 8 mM $MgCl_2$, 25 μg/mL alamethicin, 10 mM saccharic acid 1,4-lactone, 2 mM UDP-glucuronic acid (UDPGA), and HLMs or recombinant UGT isozymes (4,10). The microsomal concentration was 0.1 mg protein/mL in kinetic studies using HLMs and recombinant UGT1A1. In the study using a panel of UGT isozymes, the protein concentration was 0.2 mg protein/mL for all isozymes. The niflumic acid concentrations were 1–200 μM in kinetic studies, and 10 μM in correlation and inhibition studies of HLMs, as well as a panel study with nine UGT isozymes and inhibition study in UGT1A1. After preincubating reaction mixtures for 5 min at 37°C, the reaction was started by adding UDPGA, and reaction mixtures were incubated at 37°C for predesignated amounts of time. The reaction times were 20 and 30 min, respectively, in HLMs and recombinant UGT isozymes. Kinetic analysis was conducted by using protein concentrations and incubation times that yielded linear product formation. Incubation time (10–60 min) and protein concentration (0.1–0.5 mg protein/mL) in HLMs and UGT Supersomes™ were investigated to obtain optimum conditions. A protein concentration of 0.1 mg protein/mL and incubation times of 20 min for HLMs and 30 min for UGT1A1 were selected. The alamethicin concentration used in this study (25 μg/mL) was recommended by the supplier of the HLMs and recombinant UGT isozymes. The control activity for glucuronidation in HLMs and UGT isozymes was also determined under this alamethicin concentration by the supplier.

The reactions were terminated by the addition of acetonitrile (0.04 mL), followed by 0.01 mL of 10% formic acid (v/v), then mixtures were centrifuged at 1870 × *g* for 5 min to obtain the supernatant. Aliquots (0.1 mL) of the supernatant were injected into the HPLC equipped with an ultraviolet detector.

Identification of Glucuronide by LC–MS/MS Analysis

The incubation of niflumic acid in HLMs for structure identification was conducted as described above with some modification. Niflumic acid was incubated with HLMs and boiled HLMs in the absence and presence of UDPGA for 1 h. The reaction mixtures were further incubated in the absence and presence of β-glucuronidase (100,000 U/mL) for 3 h. Detection of the glucuronide was carried out using TSQ7000 (Thermo Finnigan, San Jose, CA, USA), an LC–MS/MS system, coupled with a Surveyor HPLC system (Thermo Finnigan). Ionization of the analytes was achieved in the positive ion mode. MS conditions were as follows: capillary voltage, 4.5 kV, capillary temperature, 300°C. MS and MS/MS spectra were obtained in the range of m/z 200 to 600 and 150 to 600, respectively. Collision-induced dissociation was performed using argon as the collision gas, and a collision energy set at −20 eV. The mobile phase consisted of 10 mM ammonium acetate–acetonitrile (7:3, v/v), and the flow rate was 0.5 mL/min. Chromatographic separation was achieved by using a reverse-phase C18 column, Capcellpak UG120 (4.6 × 150 mm, 5 μm, Shiseido, Tokyo, Japan), at a column temperature of 40°C.

Assay

The peak area of niflumic acid glucuronide was analyzed by reverse-phase HPLC (Shimadzu, Kyoto, Japan). The HPLC system consisted of LC-10AS pumps, an SCL-10A system controller, an SIL-10A autosampler, an SPD-10AV ultraviolet detector, and a C-R4AX integrator. Chromatographic separation was achieved by using a Capcellpak UG120 column (4.6 × 150 mm, Shiseido) with a flow rate of 1.0 mL/min. The mobile phase consisted of 10 mM ammonium acetate–acetonitrile (7:3, v/v) and delivered at a flow rate of 1.0 mL/min. The column elute was monitored at the ultraviolet wavelength of 290 nm. The retention times of niflumic acid glucuronide and niflumic acid were 6.4 and 9.6 min, respectively. The total run time for each injection was 13.0 min. Because of the absence of the authentic standard for niflumic acid glucuronide, quantification of the glucuronide in the reaction mixtures was conducted using a niflumic acid standard curve. The sum of the peak areas of niflumic acid and niflumic acid glucuronide after incubation of niflumic acid in the presence of UDPGA is comparable to the peak area of niflumic acid before incubation.

Chemical Inhibition

Niflumic acid glucuronidation in HLMs and recombinant UGT1A1 was measured in the absence and presence of β-estradiol. To determine the IC_{50} value of β-estradiol, niflumic acid (10 μM) was incubated in the absence and presence of β-estradiol (1–100 μM). The incubation time was 20 min and protein concentration was 0.1 mg protein/mL for both HLMs and UGT1A1. In addition, to assess the inhibition type of β-estradiol for niflumic acid glucuronidation in HLMs, niflumic acid (2–50 or 200 μM) was incubated in the absence and presence of β-estradiol (20 μM).

Time-Dependent Inhibition of UGT1A1 Activity by Niflumic Acid

The first reaction mixtures containing niflumic acid (100 and 500 μM) or 0.5% DMSO (as a control) in recombinant UGT1A1 were incubated at 37°C in the presence of UDPGA for 0 and 20 min. Aliquots (0.05 mL) of the first reaction mixtures were added to secondary reaction mixtures (5-fold dilution, with a total volume of 0.25 mL) containing 4-MU as a substrate for glucuronidation in UGT1A1. The secondary reaction was allowed to proceed for 20 min, after which it was stopped by the addition of acetonitrile, and then vortexed. The organic phase was evaporated to dryness and reconstituted in 20 mM ammonium acetate–acetonitrile (6:4, v/v). An aliquot (0.05 mL) was injected into an HPLC system, and 4-MU glucuronidation (4-MUG) was assayed as described previously (10).

Kinetic Analysis

The kinetics of niflumic acid glucuronidation in HLMs and recombinant UGT1A1 was fitted to the Michaelis-Menten kinetics with a nonsaturable component and substrate-inhibition kinetics equations (10) [Eqs. (1) and (2)], respectively, to estimate the Michaelis constant (K_m), the maximum velocity (V_{max}), and the substrate-inhibition constant (K_{si}). These fittings were carried out by using Prism Ver. 3.02 (Graph Pad Software, San Diego, CA, USA). The CL_{int}, the intrinsic

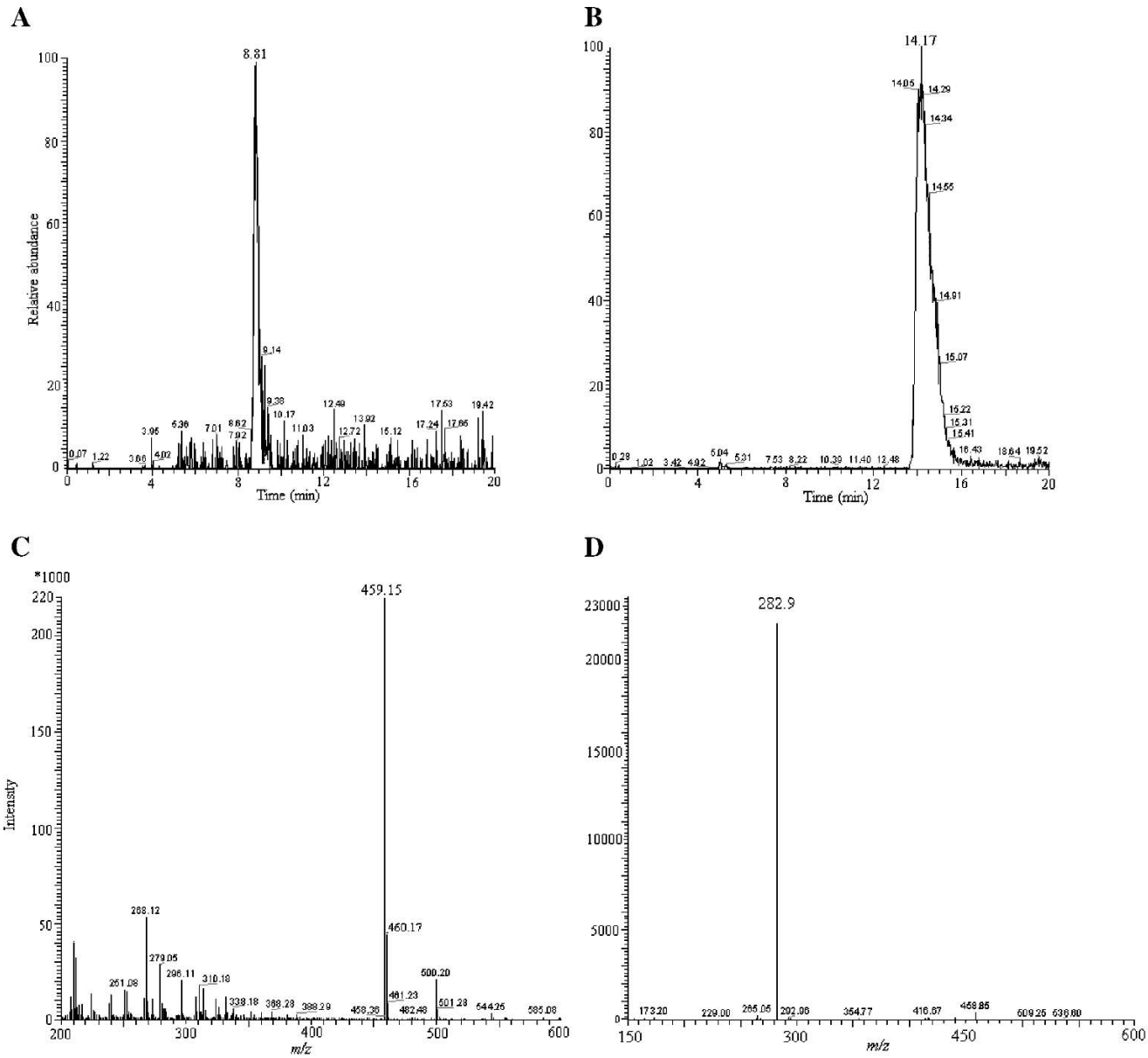

Fig. 2. LC–MS analysis of a reaction mixture following incubation of niflumic acid with UDPGA in pooled human liver microsomes. Chromatograms for niflumic acid glucuronide (A, *m*/*z* 459) and for niflumic acid (B, *m*/*z* 283) are represented. Positive ion electrospray MS (C) and MS/MS (D) spectra of the peak at the retention time of 8.8 min are also shown.

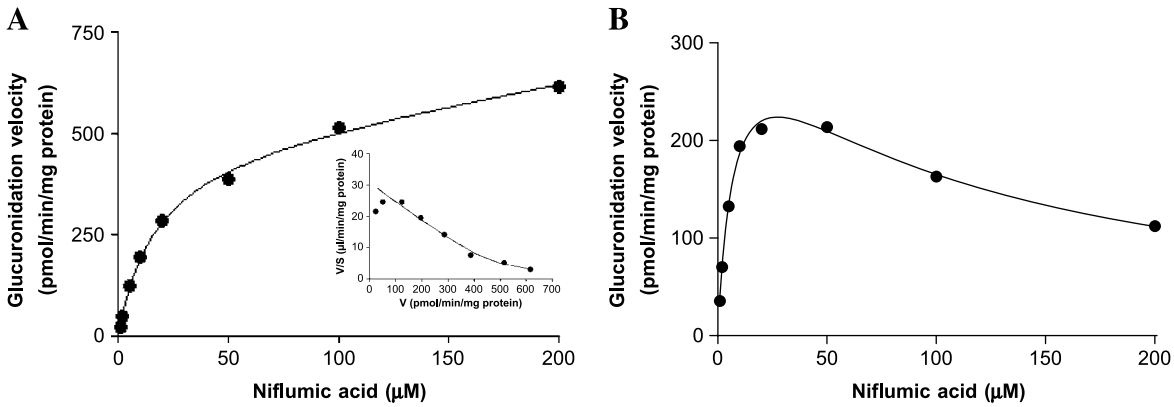

Fig. 3. Kinetics of niflumic acid glucuronidation in pooled human liver microsomes (A) and recombinant UDP-glucuronosyltransferase (UGT)1A1 (B). The Eadie–Hofstee plot is represented as an inset (A). Niflumic acid (1–200 μM) was incubated with a protein concentration of 0.1 mg protein/mL for 20 and 30 min for human liver microsomes and UGT1A1, respectively. Glucuronidation activity was determined by HPLC as described in "Materials and Methods." Each incubation was performed in duplicate.

glucuronidation clearance value, was calculated from the sum of V_{max}/K_m and a, and V_{max}/K_m for the HLMs and UGT1A1, respectively.

The K_i value of β-estradiol for niflumic acid glucuronidation was estimated by fitting the data to Eq. (3) with regression.

$$V = V_{max} \times S/(K_m + S) + a \times S \quad (1)$$

where a, defined as a nonsaturable clearance component, is the low-affinity component contributed in niflumic acid glucuronidation in HLMs.

$$V = V_{max} \times S/(K_m + S + S^2/K_{si}) \quad (2)$$

$$V = V_{max} \times S/(K_m \times (1 + I/K_i) + S) \quad (3)$$

where I is the β-estradiol concentration (20 μM).

Correlation Analysis

Correlation between niflumic acid glucuronidation and other UGT isozyme activity (UGT1A1, 1A4, and 1A9) was determined by Pearson's moment method using Prism Ver. 3.02. Glucuronidation activities for β-estradiol at the 3-OH position, trifluoperazine, and propofol in microsomes from eight human livers were used to represent typical activity for UGT1A1, 1A4, and 1A9, respectively, by the manufacturer. A p value of less than 0.05 was considered statistically significant.

RESULTS

Glucuronidation in HLMs

Incubation of niflumic acid with HLMs resulted in one peak that was observed only in the presence of UDPGA. Chromatograms for niflumic acid glucuronide (Fig. 2A, m/z 459) and for niflumic acid (Fig. 2B, m/z 283) are represented. Boiled HLMs showed no peaks with m/z 459 (data not shown). The electrospray ionization mass spectra of the peak in Fig. 2A had an $[M + H]^+$ ion at m/z 459 (Fig. 2C). The product ion spectrum of the peak showed a protonated aglycon ion at m/z 283 in the positive ion mode (Fig. 2D), suggesting the loss of glucuronic acid. This peak disappeared after further incubation of the reaction mixtures with β-glucuronidase (data not shown). These findings showed that the peak formed by the incubation of niflumic acid in the presence of UDPGA was niflumic acid glucuronide.

The kinetics of niflumic acid glucuronidation was investigated in pooled HLMs. The kinetics of glucuronidation fell into a typical Michaelis-Menten kinetics pattern with a nonsaturable clearance component. The K_m value was 16 μM, and the V_{max} and a values were 472 pmol min^{-1} mg $protein^{-1}$ and 0.91 μl min^{-1} mg $protein^{-1}$, respectively (Fig. 3A, Table I). The CL_{int} value was 31 μl min^{-1} mg $protein^{-1}$ (Table I).

Table I. Kinetic Parameters for Niflumic Acid Glucuronidation in Human Liver Microsomes (HLMs) and Recombinant UGT1A1

	K_m (μM)	V_{max} (pmol min^{-1} mg $protein^{-1}$)	K_{si} (μM)	a (μl min^{-1} mg $protein^{-1}$)	CL_{int} (μl min^{-1} mg $protein^{-1}$)
HLMs	16±2.3	472±35	N.D.	0.91±0.18	31
UGT1A1	8.0±0.95*	355±21	94±12	N.D.	45

Data represent the mean ± SE.
N.D., not determined.
*$p < 0.05$. The differences in K_m value are statistically significant.

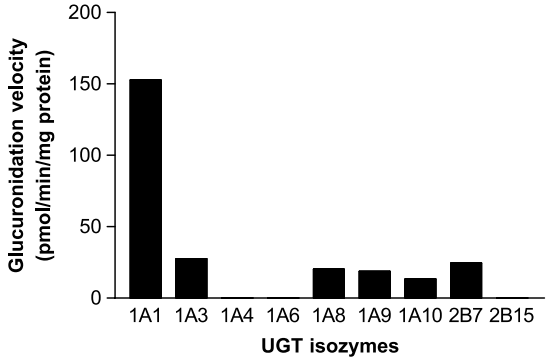

Fig. 4. Glucuronidation activities of niflumic acid in recombinant human UGT isozymes. Niflumic acid (10 μM) was incubated with UGT isozymes (0.2 mg protein/mL) for 30 min. Each incubation was performed in duplicate.

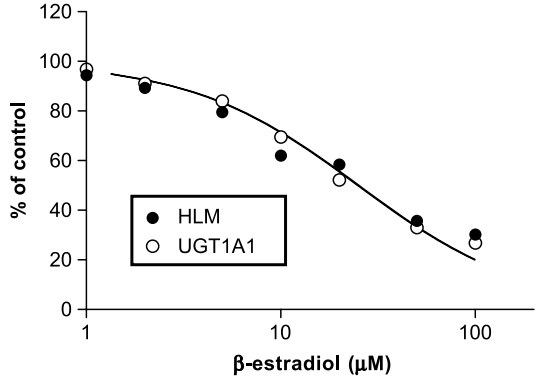

Fig. 6. Inhibitory effects of β-estradiol for niflumic acid glucuronidation in human liver microsomes and recombinant UGT1A1. Niflumic acid (10 μM) was incubated with human liver microsomes and UGT1A1 (0.1 mg protein/mL) in the absence and presence of β-estradiol (0–100 μM) for 20 min. Each incubation was performed in duplicate.

Glucuronidation in Recombinant Human UGT Isozymes

The following recombinant human UGT isozymes expressed in baculovirus-infected insect cells showed glucuronidation activity for niflumic acid: UGT1A1, 1A3, 1A8, 1A9, 1A10, and 2B7 (Fig. 4). The glucuronidation rates were 153, 28, 20, 19, 13, and 25 pmol min^{-1} mg $protein^{-1}$ for UGT1A1, 1A3, 1A8, 1A9, 1A10, and 2B7, respectively. Among these isozymes, UGT1A1 had the highest glucuronidation activity. The kinetics of niflumic acid glucuronidation was investigated in recombinant UGT1A1. The kinetics indicated substrate inhibition with K_m and K_{si} values of 8.0 and 94 μM, respectively (Fig. 3B, Table I).

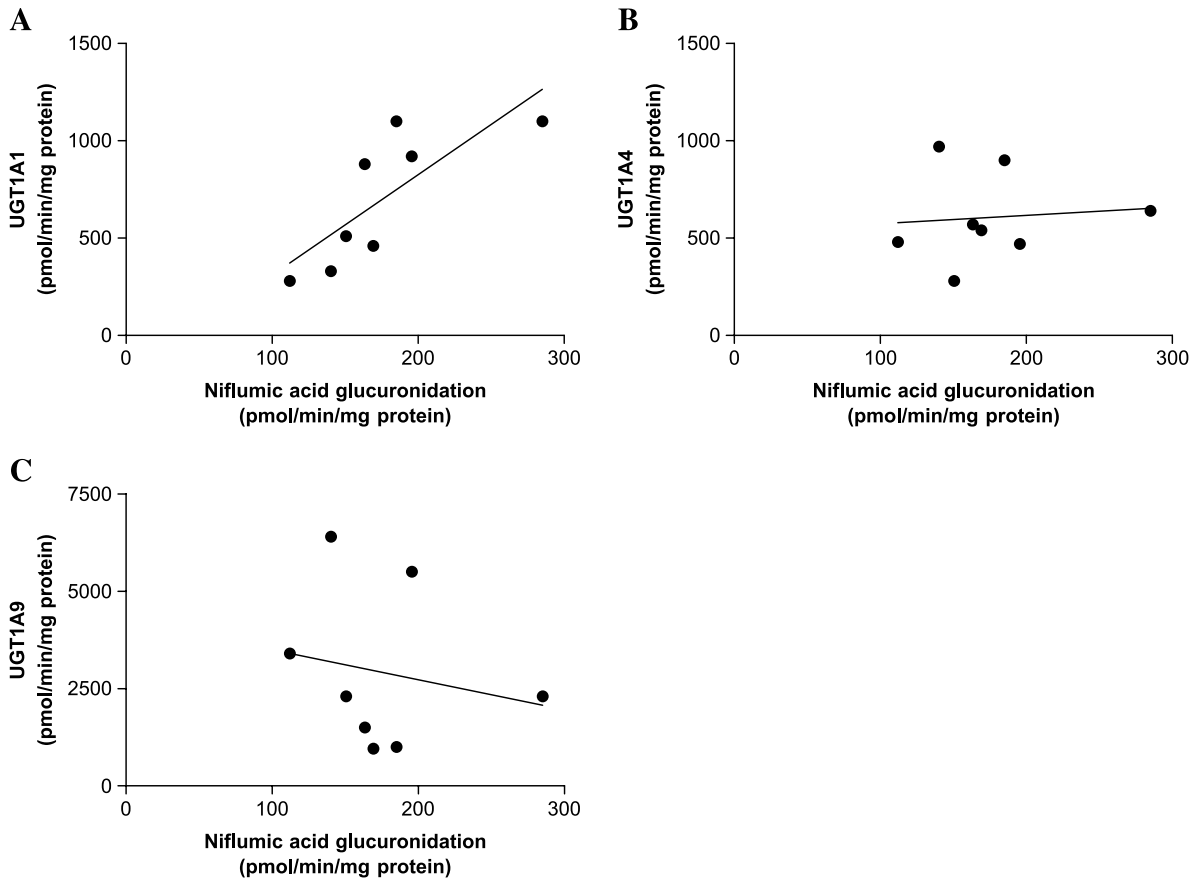

Fig. 5. Correlation analysis between niflumic acid glucuronidation and UGT isozyme-catalyzed glucuronidation in microsomes from eight human livers. Niflumic acid (10 μM) was incubated with microsomes (0.1 mg protein/mL) for 20 min. Each incubation was performed in duplicate. The *x* axis represents niflumic acid glucuronidation activity and the *y* axis represents the activity for estradiol 3β-glucuronidation (A), trifluoperazine *N*-glucuronidation (B), and propofol glucuronidation (C).

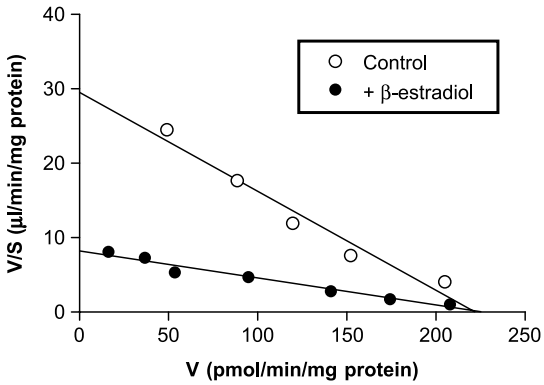

Fig. 7. Inhibition of niflumic acid glucuronidation by β-estradiol in human liver microsomes. Niflumic acid (2–50 or 200 μM) was incubated with human liver microsomes (0.1 mg protein/mL) in the absence and presence of β-estradiol (20 M) for 20 min. Each incubation was performed in duplicate.

Inter–individual Differences for Glucuronidation in HLMs and Correlation Analysis

The glucuronidation rate of niflumic acid in microsomes from eight human livers ranged from 112 to 285 pmol min^{-1} mg $protein^{-1}$. The coefficient of variation for glucuronidation rate was 29%. The correlation between the glucuronidation activities of niflumic acid and estradiol (for the 3-OH position), trifluoperazine, and propofol, was investigated (Fig. 5). The correlation between estradiol 3β-glucuronidation and niflumic acid glucuronidation was high and significant ($r = 0.78$, $p = 0.022$), whereas the glucuronidation of trifluoperazine and propofol was not significantly correlated with that of niflumic acid ($r = 0.096$, $p = 0.82$ for UGT1A4, $r = 0.19$, $p = 0.65$ for UGT1A9).

Chemical Inhibition of Glucuronidation in HLMs

The niflumic acid glucuronidation in pooled HLMs was inhibited by β-estradiol with an IC_{50} value of 25±3.3 μM. Also, β-estradiol inhibited niflumic acid glucuronidation in recombinant UGT1A1 with an IC_{50} value of 25±1.7 μM (Fig. 6). Niflumic acid glucuronidation in HLMs was inhibited by β-estradiol in a competitive manner with a K_i value of 7.6 μM (Fig. 7).

Time-Dependent Inhibition

With 0 min of incubation of the first reaction mixtures, 4-MUG in the presence of 20 and 100 μM niflumic acid was 24±2.1 and 4.7±0.31% of the control, respectively. Even with a 20-min incubation of the first reaction mixtures, 4-MUG was 22±1.8 and 4.4±0.21% of the control in 20 and 100 μM niflumic acid, respectively.

DISCUSSION

This paper describes the identification of UGT isozymes responsible for niflumic acid glucuronidation in the human liver. *In vitro* metabolism studies with mass spectrometric analysis showed that incubation of niflumic acid with HLMs in the presence of UDPGA resulted in the formation of glucuronide (Fig. 2). Niflumic acid glucuronide is considered to be an acyl glucuronide (3), formed by the esterification of carboxylic acid with glucuronic acid. However, the SRM spectrum in Fig. 2 does not indicate the position of glucuronic acid.

An Eadie-Hofstee plot shown in Fig. 3 demonstrated that there is more than one enzyme involved in niflumic acid glucuronidation in the human liver. The nonlinear regression of niflumic acid glucuronidation velocity to Michaelis-Menten equation with high- and low-affinity components was attempted; however, the kinetic parameters obtained are not reliable because of their high standard deviation. Thus, data were analyzed using Michaelis-Menten kinetics with a nonsaturable clearance component. The CL_{int} value, calculated from the sum of V_{max}/K_m and a, was determined to be 31 μl min^{-1} mg $protein^{-1}$. This finding indicates that the high-affinity component, presumably catalyzed by UGT1A1, was found to contribute significantly to the total CL_{int} value.

The glucuronidation of niflumic acid in recombinant human UGT isozymes showed that UGT1A1, 1A3, 1A8, 1A9, 1A10, and 2B7 demonstrated glucuronidation activity and that UGT1A1 had the highest glucuronidation activity (Fig. 4). Comparison of the glucuronidation activity among UGT isozymes is not sufficient, by itself, to identify the isozyme responsible for the glucuronidation of drugs in the liver. It is because the relative abundance of various UGT isozymes in the human liver remains to be investigated and to be reported. In addition, Supersomes™ have different specific activities in different UGT isoforms, and UGT protein levels of respective UGT isozyme have not been investigated by Western blot analysis. Among the UGT isozymes showing glucuronidation activity for niflumic acid, UGT1A1, 1A3, 1A9, and 2B7 were expressed in the human liver (11), which suggests the possible involvement of these isozymes in the glucuronidation of niflumic acid in the liver. Because no specific inhibitors for the respective UGT isozymes have been reported, the following approaches were investigated to evaluate the main isozymes involved in the glucuronidation of niflumic acid in the human liver. The first approach was to correlate the glucuronidation of niflumic acid with isozyme-specific glucuronidation. To date, estradiol 3β-glucuronidation, trifluoperazine *N*-glucuronidation, and propofol glucuronidation have been reported as probe reactions for UGT1A1, 1A4, and 1A9, respectively. Niflumic acid glucuronidation was significantly correlated with estradiol 3β-glucuronidation (Fig. 5). The second approach was to compare K_m values for niflumic acid glucuronidation between liver microsomes and recombinant UGT isozymes of interest. When a K_m value for a UGT isozyme is similar to that obtained for human liver microsomes, it may indicate that the isozyme is responsible for glucuronidation. Thus, based on this assumption, a kinetic study of niflumic acid glucuronidation was conducted with recombinant UGT1A1, which showed a high glucuronidation rate for niflumic acid. The kinetics of niflumic acid glucuronidation in UGT1A1 showed a substrate-inhibition characteristic with a decrease in glucuronidation rate at higher substrate concentrations. This type of atypical kinetic profile for UGT1A1-catalyzed glucuronidation has also been reported for troglitazone (12) and farnesol (13) glucuronidation in HLMs, and indomethacin glucuronidation in UGT1A1 (14). The following substrate-inhibition kinetics were also reported to occur in other UGT isozymes: 4-MUG in recombinant human UGT1A3, 1A8, and 1A9 (15); mor-

phine 3- and 6-glucuronidation in UGT2B7 (16); and diclofenac glucuronidation in UGT1A3 and 1A9 (14). Negative cooperativity in niflumic acid glucuronidation in UGT1A1 assumes the binding of more than one molecule at the enzyme activity site. To assess the other possible mechanisms involved in negative cooperativity, the time-dependent inhibition of niflumic acid glucuronidation in UGT1A1 was investigated. Although this type of study is generally used to determine whether an inhibition process is mechanism based, it may highlight the possible inhibitory potential of the metabolite in this study, which is a glucuronide. Troglitazone glucuronidation in HLMs was inhibited by troglitazone glucuronide, and the authors speculate that the metabolite inhibition may be partially responsible for the substrate-inhibition kinetics of troglitazone glucuronidation (12). As an authentic sample of niflumic acid glucuronide is not available, a direct inhibition study, as was done for troglitazone, could not be conducted. The result indicates that time-dependent inhibition for niflumic acid glucuronidation in UGT1A1 does not occur. Unfortunately, this does not shed any light on the issue of negative cooperativity. A substantial decrease in glucuronidation at higher substrate concentrations was not observed in HLMs, indicating that this effect is unique to Supersomes™. The differences in enzyme kinetic mechanisms between HLMs and UGT1A1 are not easily explainable, but some possible causes are as follows: 1) differences in phospholipid composition between HLMs and UGT1A1, 2) protein–protein interactions between CYP and UGT in HLMs (17), and 3) acyl glucuronide reactivity with UGT isozymes. However, further examination is necessary to elucidate the true cause.

The K_m value of niflumic acid in recombinant UGT1A1 was 8.0 μM (Table I). Statistical analysis revealed that the K_m value for niflumic acid glucuronidation is statistically different for UGT1A1 and HLMs (16 μM). Even when the K_m value for niflumic acid glucuronidation in recombinant UGT1A1 is similar to that in HLMs, this finding does not always lead to the conclusion that UGT1A1 is responsible for niflumic acid glucuronidation in the liver. However, the results from correlation study together with this finding support the conclusion that UGT1A1 is mainly involved in the glucuronidation of niflumic acid in the liver. This is further supported by the finding that β-estradiol inhibited niflumic acid glucuronidation in HLMs and recombinant UGT1A1 with comparable IC_{50} values (25 μM, Fig. 6), and β-estradiol competitively inhibited the high-affinity component of niflumic acid glucuronidation with a K_i value of 7.6 μM (Fig. 7).

UGT1A1, 1A3, 1A9, and 2B7 are expressed in the intestine as well as the liver, whereas UGT1A8 and 1A10 are expressed exclusively in the intestine (11). All these isozymes were catalytically active during niflumic acid glucuronidation; thus, glucuronidation in the intestine should also be taken into consideration so that the glucuronidation of orally administered drugs such as niflumic acid may be fully assessed.

In conclusion, data indicate that UGT1A1 is the main isozyme involved in the glucuronidation of niflumic acid in the human liver.

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