Glucuronidation of Antiallergic Drug, Tranilast:
Identification of Human UGT Isoforms and Effect of Its Phase I Metabolite

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Discussion: 1169 words

Abbreviations used are: UGT, UDP-glucuronosyltransferase; N-3, 4-demethyltranilast; N-4, 3-demethyltranilast; P450, cytochrome P450 enzyme; HPLC, high-performance liquid chromatography; IS, internal standard.
Tranilast is an oral anti-allergic agent widely used in Japan. Recently, in Western populations, hyperbilirubinemia induced by tranilast was suspected during clinical trials. Tranilast has been reported to be mainly metabolized to a glucuronide and a phase I metabolite, 4-demethyltranilast (N-3). In the present study, we investigated the in vitro metabolism of tranilast in human liver and jejunum microsomes and recombinant UDP-glucuronosyltransferases (UGTs). The glucuronidation of tranilast was clarified to be mainly catalyzed by UGT1A1 in human liver and intestine. The $K_m$ values of tranilast glucuronosyltransferase activity were 51.5, 50.6, and 38.0 µM in human liver microsomes, human jejunum microsomes, and recombinant UGT1A1, respectively. The $V_{max}$ values were 10.4, 42.9, and 19.7 pmol/min/mg protein in human liver microsomes, human jejunum microsomes, and recombinant UGT1A1, respectively. When the intrinsic clearance was calculated using the in vitro kinetic parameters, microsomal protein content, and weight of tissues, tranilast glucuronosyltransferase activity was 2.5-fold higher in liver than in intestine. Tranilast glucuronosyltransferase activity was strongly inhibited by bilirubin, a typical UGT1A1 substrate, and N-3, indicating that the phase I metabolite could affect the tranilast glucuronosyltransferase activity. In the case of N-3 formation, the $K_m$ and $V_{max}$ values were 37.1 µM and 27.6 pmol/min/mg protein in human liver microsomes. The bilirubin glucuronosyltransferase activity was strongly inhibited by both tranilast and N-3, suggesting that tranilast-induced hyperbilirubinemia would be responsible for the inhibition by tranilast and N-3 of the bilirubin glucuronosyltransferase activity as would the $UGT1A1$ genotype.
Introduction

Tranilast (N-(3',4'-demethoxycinnamoyl)-anthranilic acid) is an oral anti-allergic agent developed by Kissei Pharmaceutical Co. Ltd. Japan and widely used in Japan for bronchial asthma, allergic rhinitis, atopic dermatitis, keloid, and hypertrophic scar. The mechanism of its efficacy is to inhibit chemical mediators from mast cells (Azuma et al., 1976) and the accumulation of collagen in granulation tissue (Isaji et al., 1987). Recently, a clinical trial regarding the prevention of restenosis after percutaneous transluminal coronary revascularization was performed in Western populations (Holmes et al., 2000). During that trial, it was found that hyperbilirubinemia might be induced by tranilast and the risk of hyperbilirubinemia was increased in individuals with Gilbert's syndrome (Danoff et al., 2004).

Major metabolic pathways of tranilast have been shown to be glucuronidation, 4-demethylation (N-3), and sulfation of N-3 in the data sheet of tranilast provided by Kissei Pharmaceutical (Fig. 1). Tranilast, N-3, and N-3 sulfate were reported to be detected in human urine (Slobodzian et al., 1985). Since the urine sample was hydrolyzed by glucuronidase and/or base in that paper, the formation of glucuronide could be speculated by a comparison of the chromatograms before and after hydrolysis. The major metabolites of tranilast in urine in human were tranilast glucuronide and N-3 sulfate and their recoveries were almost the same (unpublished report from Kissei Pharmaceutical). Although phase I metabolism of tranilast was shown to be mainly catalyzed by cytochrome P450 2C9 (CYP2C9) in human (unpublished data from Kissei Pharmaceutical), tranilast metabolism in glucuronidation still remains uncertain.

In the case of many drugs, since a parent drug and/or its phase I metabolite could be conjugated with glucuronic acid, the role of uridine diphosphate-glucuronosyltransferase (UGT) has recently received attentions. The major metabolic pathway of a drug is not always catalyzed by P450s. In the case of tranilast, N-3 formation was catalyzed by CYP2C9 and the possibility of drug interaction with warfarin was described in the data sheet of tranilast.
However, the kinetics of tranilast glucuronidation is unknown. The purpose of the present study was to clarify the tranilast metabolism involving glucuronidation. In addition, in order to investigate the mechanism of tranilast-induced hyperbilirubinemia, the inhibitory effects of tranilast and N-3 on bilirubin glucuronosyltransferase activity were demonstrated.

**Materials and Methods**

**Materials.** Tranilast, 4-demethyltranilast, and 3-demethyltranilast (N-4) were kindly supplied by Kissei Pharmaceutical (Nagano, Japan). UDP-glucuronic acid (UDP-GA), alamethicin, β-estradiol, emodin, and β-glucuronidase from *Helix pomatia* (Type H-2) were purchased from Sigma-Aldrich (St. Louis, MO). Bilirubin, 4-nitrophenol, and imipramine hydrochloride were obtained from Wako Pure Chemicals (Osaka, Japan). Propofol was kindly provided by AstraZeneca (London, UK). Nicotinamide adenine dinucleotide phosphate (oxidized form, NADP⁺) and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast (Tokyo, Japan). 7-Hydroxycoumarin was obtained from Invitrogen (Carlsbad, CA). Pooled human liver microsomes and microsomes from 22 individual human livers were purchased from BD Gentest (Woburn, MA). The glucuronosyltransferase activity of β-estradiol in these human individual liver microsomes was provided as the typical activity for UGT1A1 by the manufacturer. The human jejunum microsomes (HJM0040) were purchased from KAC (Kyoto, Japan). Recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17 expressed in baculovirus-infected insect cells were also obtained from BD Gentest. All other chemicals and solvents were of analytical or the highest grade commercially available.

**Tranilast Glucuronidation Assay.** A typical incubation mixture (total volume, 0.2 ml) contained 50 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl₂, 50 μg of alamethicin/mg
microsomal protein, 5 mM UDP-GA, 0.5 mg/ml human liver microsomes, and tranilast. The reaction was initiated by the addition of UDP-GA and the mixture was then incubated for 60 min at 37°C. The reaction was terminated by boiling for 5 min. After removal of the protein by centrifugation at 9,000 g for 5 min, an 80 µl portion of the sample was subjected to high-performance liquid chromatography (HPLC) with a NovaPack Phenyl 4-µm analytical column (3.9 x 150 mm, Waters, Milford, MA). The product formation was measured as described previously (Slobodzian et al., 1985) with slight modifications. The mobile phase was methanol : 50 mM sodium dihydrogen phosphate (pH5.3) = 40 : 60 (v/v) and the flow rate was 1.0 ml/min. The eluent was monitored at 335 nm with a noise-base clean Uni-3 (Union, Gunma, Japan). The retention times of tranilast glucuronide, tranilast, and 7-hydroxycoumarin (internal standard, IS) were 4.4, 10.0, and 3.6 min, respectively (Fig. 2). None of these chromatograms showed any interfering peaks with tranilast glucuronide. For the quantification of tranilast glucuronide, the eluate of the HPLC from the incubation mixture with human liver microsomes, including tranilast glucuronide, was collected with reference to the retention time. A part of the eluate was incubated with 1,000 U/ml β-glucuronidase at 37°C for 24 h. The hydrolyzed tranilast glucuronide was quantified as tranilast by HPLC. Once we determined the peak area per known content of tranilast glucuronide, the ratio was applied to the calculation of the tranilast glucuronide formed in the incubation mixtures.

**Kinetic Analyses.** The kinetic studies were performed using human liver microsomes, human jejunum microsomes, and recombinant human UGT1A1 expressed in baculovirus-infected insect cells. When determining the kinetic parameters, the tranilast concentration ranged from 5 µM to 2 mM. The kinetic parameters were estimated from the fitted curves using a computer program of KaleidaGraph (Synergy Software, Reading, PA) designed for nonlinear regression analysis.
**Correlation Analyses.** The correlations between the tranilast glucuronosyltransferase activity and the other glucuronosyltransferase activities were determined by Pearson's product moment method. A $p$ value of less than 0.05 was considered statistically significant.

**Inhibition Analysis of Tranilast Glucuronosyltransferase Activity in Human Liver and Jejunum Microsomes.** As described by Watanabe et al. (2002), bilirubin (UGT1A1), ß-estradiol (UGT1A1 and UGT1A9), 4-nitrophenol (UGT1A6 and UGT1A9), imipramine (UGT1A3 and UGT1A4), emodin (UGT1A8 and UGT1A10), and propofol (UGT1A9) are typical substrates for each UGT isoform. These six substrates were investigated for their inhibitory effects on the tranilast glucuronosyltransferase activity. For the determination of the IC$_{50}$ values, the concentration of tranilast was set at 100 µM. The final concentration of the organic solvents in the reaction mixture was <2% (v/v). The tranilast glucuronosyltransferase activities in pooled human liver microsomes and human jejunum microsomes (HJM0040) at 100 µM tranilast were determined as described above.

**Inhibition of N-3 and N-4 on Tranilast Glucuronosyltransferase Activity in Human Liver and Jejunum Microsomes.** The inhibition of N-3, a phase I metabolite of tranilast, and N-4, a structural isomer of N-3, on the tranilast glucuronosyltransferase activity was also investigated in pooled human liver microsomes and individual human jejunum microsomes. For the determination of the IC$_{50}$ values, the concentration of tranilast was set at 100 µM. For the determination of the Ki values in pooled human liver microsomes, the concentrations of tranilast, N-3, and N-4 ranged from 10 to 160 µM, 0 to 150 µM, and 0 to 30 µM, respectively. The Ki values were estimated from the fitted curve using a computer program (K cat, BioMetallics, Princeton, NJ) designed for nonlinear regression analysis.

**Tranilast 4-Demethylation Assay.** A typical incubation mixture (total volume, 0.2 ml) contained 100 mM Tris-HCl buffer (pH7.4), 0.2 mg/ml human liver microsomes, an NADPH-generating system (0.5 mM NADP$^+$, 5 mM glucose-6-phosphate, 5 mM MgCl$_2$, and...
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1 U/ml glucose-6-phosphate dehydrogenase), and tranilast. The reaction was initiated by the addition of the NADPH-generating system and was then incubated for 30 min at 37°C. The reaction was terminated by adding 100 µl of ice-cold methanol. 7-Hydroxycoumarin was added as an IS. After removal of the protein by centrifugation at 9,000 g for 5 min, an 80-µl portion of the sample was subjected to HPLC. The product formation was measured using the same method for tranilast glucuronide except the mobile phase. The mobile phase was methanol : 50 mM sodium dihydrogen phosphate (pH5.3) = 33 : 67 (v/v). The retention times of N-3, tranilast, and 7-hydroxycoumarin (IS) were 9.8, 21.4, and 4.7 min, respectively. None of these chromatograms showed any interfering peaks with N-4 (data not shown).

The kinetic studies were performed using human liver microsomes. In determining the kinetic parameters, the tranilast concentration ranged from 2 to 500 µM. Kinetic parameters were estimated from the fitted curves using a computer program of KaleidaGraph designed for nonlinear regression analysis.

Inhibition of Tranilast, N-3, and N-4 on Bilirubin Glucuronosyltransferase Activity in Human Live Microsomes. A typical incubation mixture (total volume, 0.2 ml) contained 50 mM Tris-HCl buffer (pH7.4), 5 mM MgCl₂, 50 µg of alamethicin/mg microsomal protein, 2 mM UDP-GA, 0.5 mg/ml human liver microsomes, 10 µM bilirubin, and tranilast (N-3 or N-4). The reaction was initiated by the addition of UDP-GA and was then incubated for 30 min at 37°C. The reaction was terminated by adding 100 µl of ice-cold methanol. After removal of the protein by centrifugation at 9,000 g for 5 min, a 50-µl portion of the sample was subjected to high-performance liquid chromatography with a Develosil C₃₀ 5-µm analytical column (4.6 x 150 mm, Nomura Chemical, Aichi, Japan). The product formation was measured as described previously (Luquita et al., 2001) with slight modifications. The mobile phase was 55% methanol/50 mM potassium dihydrogen phosphate and the flow rate was 1.0 ml/min. The eluent was monitored at 450 nm with a noise-base clean Uni-3 (Union,
Results

Kinetics of Tranilast Glucuronosyltransferase Activity in Human Liver or Jejunum Microsomes. Kinetic analyses of tranilast glucuronidation in human liver or jejunum microsomes were performed. As shown in Figs. 3A and 3B, the kinetics of 5-500 µM tranilast fitted to the Michaelis-Menten kinetics in both human liver and jejunum microsomes. However, when the tranilast concentration exceeded 500 µM in human liver microsomes, the tranilast glucuronosyltransferase activity gradually decreased. In human jejunum microsomes, the tranilast glucuronosyltransferase activity was also decreased at 1 and 2 mM compared to 500 µM. When the apparent kinetic parameters were estimated by fitting to the Michaelis-Menten equation with the initial velocity values at 5-500 µM tranilast, the $K_m$ values in human liver and jejunum microsomes were 51.5 ± 12.8 and 50.6 ± 5.7 µM, respectively, and the $V_{max}$ values were 10.4 ± 0.8 and 42.9 ± 1.5 pmol/min/mg protein, respectively.

Tranilast Glucuronosyltransferase Activity in Recombinant UGT Isoforms. The recombinant UGT isoforms expressed in baculovirus-infected insect cells were used to determine their tranilast glucuronosyltransferase activities (Fig. 4A). UGT1A1 exhibited the highest tranilast glucuronosyltransferase activity (13.5 pmol/min/mg protein). UGT1A3, UGT1A8, UGT1A9, UGT1A10 exhibited low tranilast glucuronosyltransferase activities.

Kinetics of Tranilast Glucuronosyltransferase Activity in Recombinant UGT1A1. As shown in Fig. 4B, the kinetics of tranilast glucuronidation in recombinant UGT1A1 at 5-500 µM tranilast fitted to the Michaelis-Menten kinetics. However, when the tranilast concentration exceeded 500 µM, the tranilast glucuronosyltransferase activity gradually
decreased as in the case of human liver and jejunum microsomes. When the apparent kinetic parameters were estimated with the initial velocity values at 5 to 500 µM tranilast, the $K_m$ value was 38.0 ± 5.6 µM and the $V_{max}$ value was 19.7 ± 0.8 pmol/min/mg protein.

**Interindividual Variability of Tranilast Glucuronosyltransferase Activity from 22 Human Livers and Correlation Analyses.** The tranilast glucuronosyltransferase activities in microsomes from 22 human livers were determined at 40 µM tranilast (Fig. 5, upper part). The tranilast glucuronosyltransferase activity ranged from 1.9 pmol/min/mg protein in HG93 to 18.3 pmol/min/mg protein in HH31. The interindividual variability in the tranilast glucuronosyltransferase activity was 9.5-fold. Correlation analyses were performed between the tranilast glucuronosyltransferase activity and bilirubin (UGT1A1), ß-estradiol (UGT1A1), etoposide (UGT1A1), trifluoperazine (UGT1A4), propofol (UGT1A9), or morphine glucuronosyltransferase activities (UGT2B7) provided by the manufacturer (Table 1). The etoposide glucuronosyltransferase activities (UGT1A1) were measured in our laboratory by Watanabe et al. (2002). Since we could not obtain those activities in all individual liver microsomes, correlation analyses among bilirubin, etoposide, and morphine glucuronosyltransferase activities were performed using 11 out of 22 liver microsomes. The tranilast glucuronosyltransferase activities in individual human liver microsomes were significantly correlated with the ß-estradiol ($r=0.956$, $p<0.0001$), bilirubin ($r=0.937$, $p<0.0001$), and propofol ($r=0.449$, $p<0.05$) glucuronosyltransferase activities. The tranilast glucuronosyltransferase activities did not correlated with the trifluoperazine ($r=0.179$) and morphine ($r=0.257$) glucuronosyltransferase activities.

**Inhibition Analyses of Glucuronosyltransferase Activity in Human Liver or Jejunum Microsomes.** The inhibitory effects of bilirubin, ß-estradiol, 4-nitrophenol, imipramine, emodin, and propofol on the tranilast glucuronosyltransferase activity in human liver and jejunum microsomes were determined. As shown in Fig. 6A, the tranilast
glucuronosyltransferase activity in pooled human liver microsomes was inhibited by bilirubin (IC$_{50}$=123.9 µM). As shown in Fig. 6B, the activity in human jejunum microsomes was strongly inhibited by bilirubin (IC$_{50}$=81.1 µM) and β-estradiol (IC$_{50}$=75.3 µM).

**Inhibition of N-3 and N-4 on Tranilast Glucuronosyltransferase Activity in Human Liver and Jejunum Microsomes.** The inhibitory effects of N-3 and N-4 on the tranilast glucuronosyltransferase activity in human liver and jejunum microsomes were determined. The tranilast glucuronosyltransferase activities in human liver and jejunum microsomes were strongly inhibited by both N-3 and N-4 (Fig. 6). The IC$_{50}$ values of N-3 and N-4 were 141.7 and 81.3 µM, respectively in liver microsomes and 82.8 and 45.9 µM, respectively in jejunum microsomes. The inhibition pattern of N-3 was competitive. The $K_{is}$ value of N-3 for the tranilast glucuronosyltransferase activity in human liver microsomes was 52.8 µM (Fig. 7). On the other hand, the inhibition pattern of N-4 was mixed. The $K_{is}$ and $K_{ii}$ values of N-4 for the tranilast glucuronosyltransferase activity in human liver microsomes were 42.6 and 181.1 µM, respectively (Fig. 7).

**Kinetics of Tranilast 4-Demethylase Activity in Human Liver Microsomes.** The kinetics of tranilast 4-demethylation (N-3 formation) in pooled human liver microsomes at 2-500 µM tranilast fitted to the Michaelis-Menten kinetics. The $K_m$ and $V_{max}$ values were 37.1 ± 3.1 µM and 27.6 ± 0.7 pmol/min/mg protein, respectively.

**Interindividual Variability of Tranilast 4-demethylase Activity from 22 Human Livers and Correlation Analyses.** The tranilast 4-demethylase activities in microsomes from 22 human livers were determined at 40 µM tranilast. The interindividual variability in the tranilast glucuronosyltransferase activity was at most 16.4-fold (Fig. 5, lower part). The tranilast 4-demethylase activity ranged from 4.5 pmol/min/mg protein in HG32 to 73.1 pmol/min/mg protein in HG30. Correlation analyses were performed between the tranilast 4-demethylase activity and phenacetin O-deethylation activity (CYP1A2), coumarin
7-hydroxylase activity (CYP2A6), S-mephenytoin N-demethylase activity (CYP2B6),

paclitaxel 6α-hydroxylase activity (CYP2C8), diclofenac 4'-hydroxylase activity (CYP2C9),

S-mephenytoin 4'-hydroxylase activity (CYP2C19), bufuralol 1'-hydroxylase activity
(CYP2D6), chloroxazone 6-hydroxylase activity (CYP2E1), testosterone 6ß-hydroxylase
activity (CYP3A4), or lauric acid 12-hydroxylase activity (CYP4A) provided by the
manufacturer. The tranilast 4-demethylase activities in the 22 human liver microsomes were
significantly correlated with the diclofenac 4'-hydroxylase activities (r=0.825, p<0.0001) and
the paclitaxel 6α-hydroxylase activities (r=0.576, p<0.01).

Inhibition of Tranilast, N-3, and N-4 on Bilirubin Glucuronosyltransferase Activity in
Human Live Microsomes. The inhibitory effects of tranilast, N-3, and N-4 on the bilirubin
glucuronosyltransferase activity in human liver microsomes were determined. The bilirubin
glucuronosyltransferase activity was strongly inhibited by tranilast, N-3 and N-4. The IC₅₀
values of tranilast, N-3, and N-4 were 28.7, 76.9, and 62.0 µM, respectively.

Discussion

Although tranilast has been prescribed for many decades in Japan, its metabolism has not
been investigated completely. In human, the major metabolites have been reported to be
tranilast glucuronide and N-3 sulfate (unpublished data, Kissei Pharmaceutical). In human
urine, other metabolites such as N-2

(2-(4'-hydroxy-3’-methoxystyryl)-3,1-benzoxazin-4-one), N-6 (1-benzoxazin-4-one), and N-3
glucuronide (unpublished data, Kissei Pharmaceutical) were slightly detected. In the present
study, it was clarified that tranilast glucuronidation was mainly catalyzed by UGT1A1 in
human. UGT1A3, UGT1A8, and UGT1A10 were partly responsible for the tranilast
glucuronidation. The tranilast glucuronosyltransferase activity could be detected in both
human liver and jejunum microsomes. UGT1A1 is one of the major isoforms of UGTs in the
liver, and is also expressed in the intestine. UGT1A8 and UGT1A10 could be responsible for the extrahepatic metabolism of tranilast because these isoforms are expressed in the intestine but not in liver. However, the tranilast glucuronosyltransferase activity in human jejunum microsomes was inhibited by bilirubin and β-estradiol but not by emodin (Fig. 6B), suggesting that the tranilast glucuronosyltransferase activity was mainly catalyzed by UGT1A1 in intestine as in the liver.

The tranilast glucuronosyltransferase activity was reduced at high substrate concentrations (>500 µM) in human liver and jejunum microsomes and recombinant UGT1A1. The reason for this phenomenon was unclear, but substrate and/or metabolite inhibition may be involved. Further study is needed to clarify the mechanism of the inhibition. The maximum serum concentration of tranilast in human has been reported to be 37.0 µM after single oral administration at the therapeutic dose of 100 mg (Slobodzian et al., 1985). As reported by Kissei Pharmaceutical, the maximum serum concentration of tranilast was 67.8 µM after taking 2.5 mg/kg tranilast three times per day for 5 days. In clinical practice, the concentration of tranilast is unlikely to reach 500 µM. Therefore, the kinetic parameters fitted to the Michaelis-Menten equation with <500 µM tranilast seems to be reasonable.

The in vitro intrinsic clearance (CL_{int}) is calculated using the following equation (Obach et al., 1997; Soars et al., 2002):

\[
CL_{int} = \frac{V_{max}}{K_m} \times \frac{\text{[microsomal protein/tissue (mg/g)]}}{\text{[tissue/body weight (g/kg)]}}.
\]

Soars et al. (2002) reported that there were 45 mg of microsomal protein/g of liver and 20 g of liver/kg of body weight. The CL_{int} in liver of tranilast was calculated to be 181.7 µl/min/kg. They also reported that there are 3 mg of microsomal protein/g of intestine and 30 g of intestine/kg of body weight (Soars et al., 2002). The CL_{int} in intestine was estimated to be 76.3 µl/min/kg. The glucuronosyltransferase activity has been reported to differ according to regions of the intestine in human (Strassburg et al., 2000). The UGT1A1 activity in human
was higher in upper intestine than lower intestine (Basu et al., 2004). Although the UGT activity in the intestine may differ from that in the liver, the tranilast glucuronosyltransferase activity in the intestine might be approximately 40% of that in the liver. UGT1A1 has shown polymorphic metabolism. Particularly, the relationship between the toxicity of irinotecan hydrochloride and the \textit{UGT1A1} genotype has been extensively studied (Ando et al., 2005). As well as irinotecan hydrochloride, \textit{UGT1A1} genetic polymorphism would affect the tranilast pharmacokinetics.

Another metabolic pathway of tranilast is N-3 formation catalyzed by CYP2C9 (unpublished data, Kissei Pharmaceutical). We firstly analyzed the kinetics of tranilast 4-demethylase activity in human liver microsomes. The $K_m$ value of N-3 formation in human liver microsomes was $37.1 \pm 3.1 \mu$M, which was similar to that of the tranilast glucuronosyltransferase activity. There was large interindividual variability (>16-fold) in the N-3 formation. In CYP2C9, several polymorphic alleles have been reported to have decreased enzyme activity in vitro and in vivo (Takahashi et al., 1998; Blaisdell et al., 2004). The frequency of poor metabolizing alleles such as \textit{CYP2C9}*2 and \textit{CYP2C9}*3 was different between ethnic groups (Sullivan-Klose et al., 1996; Blaisdell et al., 2004). Therefore, the genetic polymorphisms of \textit{CYP2C9} and \textit{UGT1A1} would play important roles in the pharmacokinetics of tranilast.

In the present study, N-3 was demonstrated to inhibit the tranilast glucuronosyltransferase activity. Although the concentration of N-3 in the liver is unknown, N-3 could affect the tranilast glucuronosyltransferase activity, leading to altered pharmacokinetics of tranilast. N-3 is further metabolized to be N-3 glucuronide and could be slightly detected in human urine (unpublished data, Kissei Pharmaceutical). Since the inhibition pattern of N-3 on the tranilast glucuronosyltransferase activity was competitive, the glucuronidation of N-3 might be catalyzed by UGT1A1. N-4, a structural isomer of N-3, also inhibited the tranilast
glucuronosyltransferase activity. However, there are no reports that N-4 could be detected in human. Because glucuronidation of a drug may be inhibited by its P450 metabolites, the inhibition by metabolites should be keep in mind when estimating the pharmacokinetics.

Recently, the PRESTO (prevention of restenosis with tranilast and its outcomes) study was performed in Western populations since tranilast may have a benefit in preventing restenosis after percutaneous transluminal coronary revascularization (Holmes et al., 2000). During the phase III clinical trial, an increase in serum unconjugated bilirubin was observed in 12% of white subjects (Danoff et al., 2004). It was suspected that tranilast-induced hyperbilirubinemia might be caused by the inhibition by tranilast and N-3 of the bilirubin glucuronosyltransferase activity. Danoff et al. (2004) suggested that the tranilast-induced hyperbilirubinemia might be related to genetic polymorphisms of \textit{UGT1A1}. As reported by Kissei Pharmaceutical, the frequencies of liver dysfunction and jaundice caused by tranilast were 0.62 and 0.008\%, respectively in Japanese. The frequency of hyperbilirubinemia in Japanese is unknown but seems to be lower than that in white subjects. The reason for this phenomenon may be interethnic variability in the \textit{UGT1A1} allelic frequency. In the case of \textit{UGT1A1}*28, which is mainly responsible for Gilbert's syndrome, its allelic frequency has been shown to be 35.7-41.3\% in whites (Monaghan et al., 1996 and 1997) but 13.8\% in Asians (Ando et al., 1998). In the patients of Gilbert's syndrome, the unconjugated bilirubin concentration may be significantly elevated after tranilast administration because of the inhibition of UGT1A1 activity by tranilast and N-3. In addition, as mentioned above, the allele frequency of the \textit{CYP2C9} poor metabolizer was higher in whites than in Japanese (Nasu et al., 1997; Scordo et al., 2001). Thus, the genetic risk for the elevation of tranilast concentration would be higher in whites than in Japanese. The occurrence of adverse reactions from tranilast might be explained by the enzyme inhibition and the genetic polymorphisms of both phase I and phase II enzymes.
In conclusion, it was clarified that the tranilast glucuronosyltransferase activity was mainly catalyzed by UGT1A1 in human liver and intestine. N-3, a phase I metabolite of tranilast, inhibited the tranilast glucuronosyltransferase activity, suggesting that the inhibition by a phase I metabolite may be noteworthy when estimating the glucuronidation of a drug. The inhibition by tranilast and N-3 of the bilirubin glucuronosyltransferase activity may be partly responsible for the tranilast-induced hyperbilirubinemia. We should keep in mind that UGT1A1 substrates may inhibit the bilirubin glucuronosyltransferase activity leading to hyperbilirubinemia, and that phase I metabolites can affect the glucuronidation of its parent drug.

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Footnotes

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Legends for Figures

FIG. 1. Metabolic pathways of tranilast in human.

FIG. 2. Representative HPLC chromatograms of tranilast glucuronide in human liver microsomes. Tranilast was incubated at 37°C for 60 min with (A) or without (B) UDP-GA. Peak 1, 7-hydroxycoumarin (IS); 2, tranilast glucuronide; 3, tranilast.

FIG. 3. Kinetic analyses of tranilast glucuronosyltransferase activity in human liver (A) or jejunum (B) microsomes. The solid line represents the fitting curve to the Michaelis-Menten equation (5-500 µM, closed circles). The tranilast glucuronosyltransferase activity was determined as described in Materials and Methods. Each data point represents the mean of duplicate determinations.

FIG. 4. Tranilast glucuronosyltransferase activity in recombinant human UGTs in baculovirus-infected insect cells (A) and kinetic analysis in UGT1A1 (B). A, The concentration of tranilast was 100 µM. Each column represents the mean of duplicate determinations. ND: not detected. B, The solid line represents the fitting curve to the Michaelis-Menten equation (5-500 µM, closed circles). Each data point represents the mean of duplicate determinations.

FIG. 5. Interindividual variability of tranilast glucuronosyltransferase activity (A) and 4-demethylase activity (B) in microsomes from 22 human livers. A and B, The concentration of tranilast was 40 µM. Each column represents the mean of duplicate determinations.
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FIG. 6. Inhibitory effects of typical substrates for UGT isoforms, N-3, and N-4 on tranilast glucuronosyltransferase activity in human liver (A) or jejunum (B) microsomes. Bilirubin (UGT1A), β-estradiol (UGT1A1 and UGT1A9), imipramine (UGT1A3 and UGT1A49, 4-nitrophenol (UGT1A6 and UGT1A9), emodin (UGT1A8 and UGT1A10), propofol (UGT1A9), N-3, and N-4 were used as inhibitors. Each data point represents the mean of duplicate determinations. The control activity was 6.2 pmol/min/mg protein in human liver microsomes and 24.0 pmol/min/mg protein, respectively.

FIG. 7. Inhibitory effect of N-3 (A) and N-4 (B) on tranilast glucuronosyltransferase activity in human liver microsomes. Human liver microsomes were incubated with tranilast in the absence or presence of N-3 and N-4 described in Materials and Methods. Each data point represents the mean of duplicate determinations.
TABLE 1

*Correlation between tranilast glucuronosyltransferase activity and other glucuronosyltransferase activities in individual human liver microsomes*

<table>
<thead>
<tr>
<th>Glucuronidation</th>
<th>n</th>
<th>Substrate</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin glucuronidation</td>
<td>11</td>
<td>10</td>
<td>0.963</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ß-Estradiol 3-glucuronidation</td>
<td>22</td>
<td>100</td>
<td>0.965</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Etoposide glucuronidation</td>
<td>11</td>
<td>400</td>
<td>0.829</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Trifluoperazine glucuronidation</td>
<td>22</td>
<td>100</td>
<td>0.179</td>
<td>NS</td>
</tr>
<tr>
<td>Propofol glucuronidation</td>
<td>22</td>
<td>30</td>
<td>0.449</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Morphine 3-glucuronidation</td>
<td>11</td>
<td>250</td>
<td>0.257</td>
<td>NS</td>
</tr>
</tbody>
</table>

n, number of individual human liver microsomes; r, correlation coefficient; NS, not significant.
Tranilast glucuronide

Fig. 1.
Fig. 3.

A

Tranilast glucuronosyltransferase activity (pmol/min/mg protein) vs. Tranilast (μM)

B

Tranilast glucuronosyltransferase activity (pmol/min/mg protein) vs. Tranilast (μM)
**Fig. 4.**

A. Bar graph showing tranilast glucuronosyltransferase activity (pmol/min/mg protein) for different UGT enzymes (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, UGT2B17). ND indicates not determined.

B. Graph showing tranilast glucuronosyltransferase activity (pmol/min/mg protein) as a function of tranilast concentration (μM). The curve shows an increase in activity with increasing tranilast concentration.
**Fig. 5.**

![Graph showing Tranilast glucuronosyltransferase activity and Tranilast 4-demethylase activity for human liver microsomes.

Human liver microsomes

HG3, HG23, HG30, HG32, HG36, HG64, HG70, HG89, HG93, HG95, HH1, HH9, HH13, HH18, HH31, HH35, HH47, HH89, HK23.
Fig. 6.

A

Tranilast glucuronosyltransferase activity (% of control)

Inhibitor (μM)

- Bilirubin
- β-Estradiol
- Imipramine
- 4-Nitrophenol
- Emodin
- Propofol
- N-3
- N-4

B

Tranilast glucuronosyltransferase activity (% of control)

Inhibitor (μM)

- Bilirubin
- β-Estradiol
- Imipramine
- 4-Nitrophenol
- Emodin
- Propofol
- N-3
- N-4