0090-9556/06/3403-471–476$20.00
DRUG METABOLISM AND DISPOSITION
Vol. 34, No. 3
6445/3099206
DMD 34:471–476, 2006
Printed in U.S.A.
Copyright © 2006 by The American Society for Pharmacology and Experimental Therapeutics

CHARACTERIZATION OF HUMAN CYTOCHROME P450 ENZYMES INVOLVED IN THE METABOLISM OF THE PIPERIDINE-TYPE PHENOTHIAZINE NEUROLEPTIC THIORIDAZINE

Jacek Wójcikowski, Patrick Maurel, and Władysława A. Daniel

Polish Academy of Sciences, Institute of Pharmacology, Kraków, Poland (J.W., W.A.D.); and INSERM, U632, F-3493 Montpellier, France; Univ. Montpellier-I, Montpellier, F-34000 France (P.M.)

Received July 5, 2005; accepted November 2, 2005

ABSTRACT:

The aim of the present study was to identify human cytochrome P450 enzymes (P450s) involved in mono-2-, di-2-, and 5-sulfoxidation, and *N*-demethylation of the piperidine-type phenothiazine neuroleptic thioridazine in the human liver. The experiments were performed in vitro using cDNA-expressed human P450s (Supersomes 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4), liver microsomes from different donors, and P450-selective inhibitors. The results indicate that CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulfoxidation and *N*-demethylation (34–52%), whereas CYP2D6 is the basic enzyme that catalyzes mono-2- and di-2-sulfoxidation of thioridazine in human liver (49 and 64%, respectively). Besides CYP2D6, CYP3A4 contributes to a noticeable degree to thioridazine mono-2-sulfoxidation (22%). Therefore, the sulforidazine/mesoridazine ratio may be an additional and more specific marker than the mesoridazine/thioridazine ratio for assessing the activity of CYP2D6. In contrast to promazine and perazine, CYP2C19 insignificantly contributes to the *N*-demethylation of thioridazine. Considering serious side-effects of thioridazine and its 5-sulfoxide (cardiotoxicity), as well as strong dopaminergic D_2 and noradrenergic α_1 receptor-blocking properties of mono-2- and di-2-sulfoxides, the obtained results are of pharmacological and clinical importance, in particular, in a combined therapy. Knowledge of the catalysis of thioridazine metabolism helps to choose optimum conditions (a proper coadministered drug and dosage) to avoid undesirable drug interactions.

Thioridazine, a prototypic agent for phenothiazine neuroleptics of the piperidine type, is a mild neuroleptic which acts on positive and negative symptoms of schizophrenia, displaying sedative and antidepressant effects. Because of its psychotropic profile, thioridazine is suitable for combination with antidepressants in the therapy of many psychiatric disorders (psychotic depression, "treatment-resistant" depression, depression in the course of schizophrenia, schizoaffective psychosis). However, the main side effects of thioridazine are related to cardiac muscle conduction and anticholinergic activity. Among neuroleptic drugs, thioridazine produces the most distinct ECG abnormalities, which are dose-dependent (Axelsson 1977; Gottschalk et al., 1978; Llerena et al., 2002). Heiman (1977) reported cases of life-threatening ventricular arrhythmia in patients who had ingested a combination of thioridazine and imipramine or amitriptyline, alerting clinicians to the risk of using thioridazine at high doses and in combinations with tricyclic antidepressants. Therefore, knowledge of thioridazine metabolism seems to be of importance.

Like other phenothiazine neuroleptics, thioridazine undergoes *S*-oxidation in the thiazine ring in position 5, as well as aromatic hydroxylation (mainly in position 7), *N*-demethylation, and *N*-oxidation (Papadopoulos et al., 1985; Svendsen and Bird, 1986; Lin et al., 1993). However, unlike other phenothiazines, thioridazine forms a sulfoxide in position 2 of the thiomethyl substituent (mesoridazine), which is further oxidized to a sulfone (sulforidazine) (Fig. 1). Metabolites formed by *S*-oxidation in position 2, i.e., mesoridazine and sulforidazine, are more potent than thioridazine in blocking dopaminergic D_2 and noradrenergic α_1 receptors; moreover, *N*-desmethylthioridazine retains affinity for α_1 receptors (Axelsson, 1977; Bylund, 1981; Richelson and Nelson, 1984; Hyttel et al., 1985). Thioridazine 5-sulfoxide (a ring sulfoxide) is not pharmacologically active at dopaminergic or noradrenergic receptors, but is considered to contribute to the cardiotoxicity of the parent compound (Gottschalk et al., 1978; Hale and Poklis, 1986).

It is still unclear which enzymes are responsible for particular metabolic steps of thioridazine. Clinical studies demonstrated that the metabolism of thioridazine was under genetic control of hepatic CYP2D6 catalyzing 2-sulfoxidation of the neuroleptic (Meyer et al., 1990; Llerena et al., 2000, 2001). Moreover, Llerena et al. (2000) suggested that the mesoridazine/thioridazine ratio might be a useful tool to assess CYP2D6 activity. Other clinical studies showed that the plasma concentrations of thioridazine in psychiatric patients were influenced by tobacco smoking (CYP1A2 inducer) and CYP2D6 genotype, but not by the CYP2C9 genotype, which suggested that CYP1A2 in addition to CYP2D6 was involved in the metabolism of thioridazine (Berecz et al., 2003).

This study was supported by the statutory funds of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.105.006445.

ABBREVIATIONS: P450, cytochrome P450; DDC, diethyldithiocarbamic acid; HPLC, high performance liquid chromatography; K_m, the Michaelis constant; V_{max}, maximum velocity of the reaction.

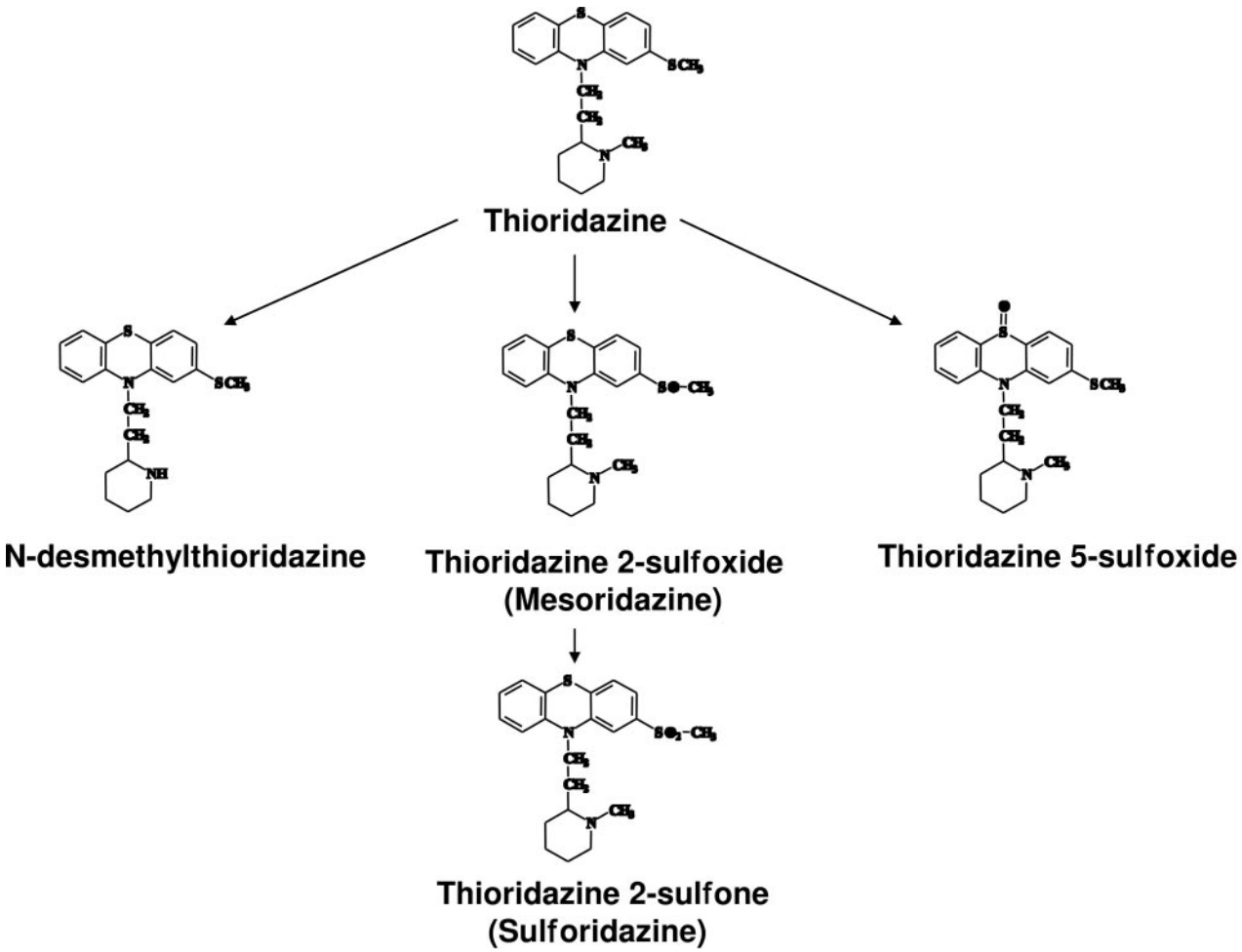


FIG. 1. The main metabolic pathways of thioridazine.

Thus, there are no complete data on the enzymatic catalysis of thioridazine metabolism in humans. Many other P450 enzymes that might be involved in the mono-2-sulfoxidation of thioridazine, as well as the catalysis of other metabolic pathways of this neuroleptics, such as di-2-sulfoxidation, *N*-demethylation, and 5-sulfoxidation processes, have not been studied in humans so far. Considering the serious side effects of thioridazine and the risk of its application in a combination therapy, in the present study, we aimed to concurrently investigate the contribution of human P450s to thioridazine mono-2-, di-2-, and 5-sulfoxidation, and *N*-demethylation using different in vitro models.

The obtained results indicate that CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulfoxidation and *N*-demethylation, whereas CYP2D6 is the key enzyme that catalyzes mono-2- and di-2-sulfoxidation of thioridazine in human liver; besides CYP2D6, CYP3A4 significantly contributes to thioridazine mono-2-sulfoxidation. The above results are compared with the findings of analogous experiments with other phenothiazine neuroleptics and are discussed with respect to the substrate-structure differences in the enzymatic catalysis of the metabolism of phenothiazines. Moreover, pharmacological and clinical aspects of the obtained results are emphasized.

Materials and Methods

Drugs and Chemicals. Thioridazine hydrochloride was obtained from Jelfa (Jelenia Góra, Poland). Mesoridazine and sulforidazine (free bases) were donated by Sandoz Pharma AG (Basel, Switzerland). Thioridazine 5-sulfoxide and *N*-desmethylthioridazine were synthesized in our laboratory as described previously (Daniel et al., 1997). α-Naphthoflavone, diethyldithiocarbamic acid (DDC), quinidine, sulfaphenazole, ticlopidine, ketoconazole, and NADPH were purchased from Sigma (St. Louis, MO). All the organic solvents with HPLC purity were supplied by Merck (Darmstadt, Germany).

Human Liver Microsomes. Human liver microsome preparations (HK23, HK25, HK37, HG03, HG43, HG56, HG89, HG93) were obtained from BD Gentest (Woburn, MA).

Liver microsomes from patients HK23, HG43, and HG89 were used for optimizing the conditions of thioridazine metabolism. Microsomal protein, 250 μg, was resuspended in 500 μl of 20 mM Tris/HCl buffer (pH = 7.4). To determine enzyme kinetic parameters, the thioridazine concentrations used ranged from 5 to 250 μM. For studies of thioridazine metabolism in individual patients, 25 μM thioridazine was used. For inhibition studies, 25 μM thioridazine was incubated with the selective P450 inhibitors: 2 μM α-naphthoflavone (a CYP1A2 inhibitor), 200 μM DDC (a CYP2A6 + CYP2E1 inhibitor), 10 μM sulfaphenazole (a CYP2C9 inhibitor), 5 μM ticlopidine (a CYP2C19 inhibitor), 10 μM quinidine (a CYP2D6 inhibitor), and 2 μM ketoconazole (a CYP3A4 inhibitor). After a 3-min preincubation at 37°C, the reaction was initiated by adding NADPH to a final concentration of 1 mM. After a 25-min incubation, the reaction was stopped by adding 200 μl of methanol. Thioridazine and its metabolites were analyzed by an HPLC method as described below.

Correlation Analysis of the Data. The rates of thioridazine mono-2-, di-2-, and 5-sulfoxidation, and *N*-demethylation were correlated with the rates of P450-specific reactions: phenacetin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), *S*-mephenytoin *N*-demethylation (CYP2B6), diclofenac 4′-hydroxylation (CYP2C9), *S*-mephenytoin 4′-hydroxylation (CYP2C19), bufuralol 1′-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), and testosterone 6β-hydroxylation. Each data pair was

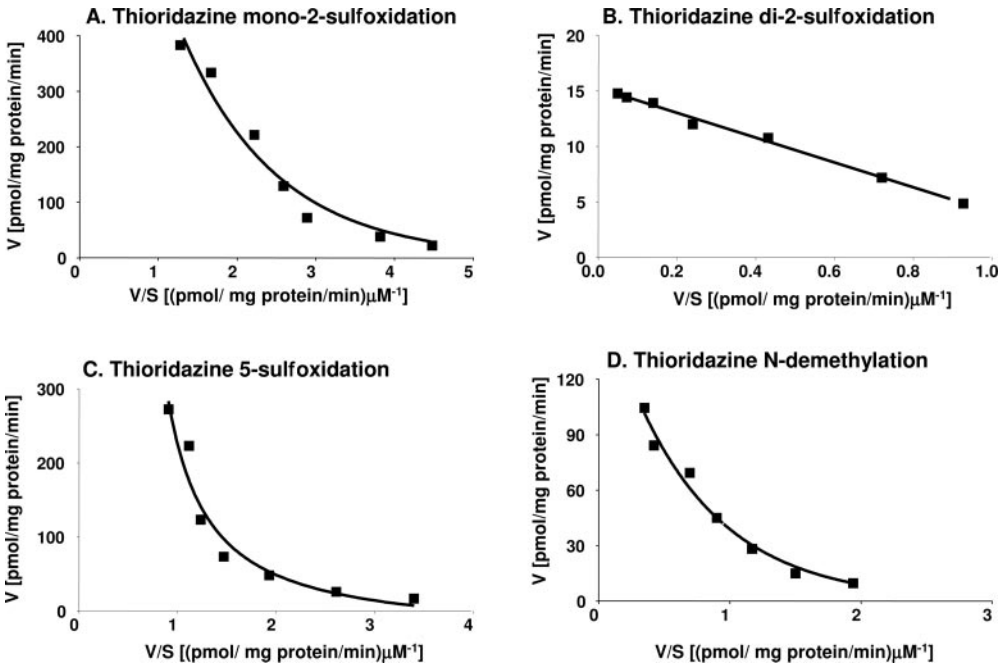


FIG. 2. Eadie-Hofstee plots for thioridazine mono-2-sulfoxidation (A), di-2-sulfoxidation (B), 5-sulfoxidation (C), and *N*-demethylation (D) in human liver microsomes. Human liver microsomes of patient HK23 (0.5 mg protein/ml) were incubated in a 20 mM Tris/HCl buffer (pH = 7.4) with thioridazine (5–250 μM) and NADPH (1 mM) for 25 min. Similar results were obtained with microsomes of patients HG43 and HG89.

compared by a simple linear regression analysis using the statistical program Prism 2.01 (GraphPad Software Inc., San Diego, CA). Monooxygenase activities for each liver microsomal preparation (donor) were determined and provided by BD Gentest.

cDNA-Expressed Human P450s. Microsomes from baculovirus-infected insect cells expressing CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 (Supersomes) were obtained from Gentest Co. (Woburn, MA., USA). Thioridazine metabolism was studied under experimental conditions similar to those described for liver microsomes, with 10, 25, 50,100 and 250 μM thioridazine, except for the fact that the final concentration of P450s was 100 pmol/ml. Thioridazine and its metabolites were analyzed by HPLC as described below.

Determination of Thioridazine and Its Metabolites in the Incubation Medium. Thioridazine and its metabolites were quantified using the previously described HPLC method (Daniel et al., 1997). After incubation, the samples were centrifuged for 10 min at 2000*g*. The water phase containing thioridazine and its metabolites was extracted (pH = 12) with hexane and 1.5% isoamyl alcohol. The residue obtained after evaporation of the microsomal extracts was dissolved in 100 μl of the mobile phase described below. An aliquot of 20 μl was injected into the HPLC system. The concentrations of thioridazine and its metabolites (mesoridazine, sulforidazine, thioridazine 5-sulfoxide, *N*-desmethylthioridazine) were assayed using a LaChrom (Merck-Hitachi) HPLC system with fluorescence detector. The analytical column (Econosphere C18, 5 μm, 4.6 $\times$ 250 mm) was purchased from Alltech (Carnforth, UK). The mobile phase consisted of an acetate buffer, pH = 3.4 (100 mmol of ammonium acetate, 20 mmol of citric acid, and 1 ml of triethylamine in 1000 ml of the buffer, adjusted to pH = 3.4 with an 85% phosphoric acid), and acetonitrile in the proportion 50:50. The flow rate was 1.5 ml min^{-1}; the column temperature was ambient. The fluorescence was measured at a wavelength of 270 nm (excitation) and 467 nm (emission) for thioridazine, mesoridazine, sulforidazine, and *N*-desmethylthioridazine, and 274/388 nm for thioridazine 5-sulfoxide. The compounds were eluted in the following order: sulforidazine (5.49 min), mesoridazine (7.82 min), diastereoisomers of thioridazine 5-sulfoxide (9.06 min and 10.27 min), *N*-desmethylthioridazine (12.86 min), and thioridazine (16.81 min). The sensitivity of the HPLC method allowed us to quantify thioridazine as low as 0.004 nmol/ml, mesoridazine and thioridazine 5-sulfoxide as low as 0.002 nmol/ml, and sulforidazine and *N*-desmethylthioridazine as low as 0.001 nmol/ml microsomal suspension. The reproducibility coefficient of variation was under 3%.

Results

Thioridazine Metabolism in Human Liver Microsomes. Figure 2, A to D, shows the representative Eadie-Hofstee plots for thioridazine oxidation processes in liver microsomes from the patient HK23. All the plots were nonlinear, except for the plot for thioridazine di-2-sulfoxidation. Similar results were obtained with liver microsomes from patients HG43 and HG89.

Correlation Study. Interindividual variability of the rates of thioridazine metabolism ranged from 2.5-fold (*N*-demethylation) to 3.9-fold (5-sulfoxidation) (Fig. 3).

The rates of formation of thioridazine metabolites by different preparations of human liver microsomes (Fig. 3) were compared with the monooxygenase activities determined for each liver preparation by the supplier (BD Gentest). The results of those analyses are shown in Table 1, where the correlation coefficient (*r*) and the *p* value are given for each pair of data. The formation of thioridazine 5-sulfoxide and *N*-desmethylthioridazine was significantly correlated with phenacetin

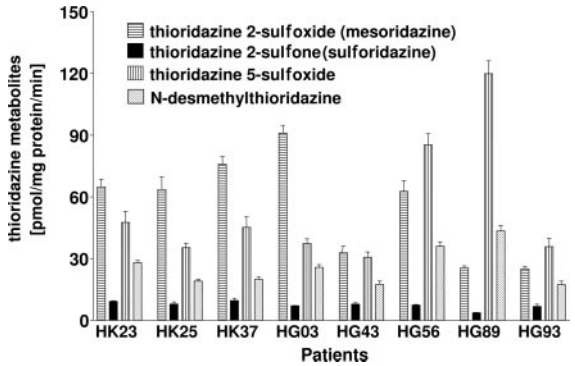


FIG. 3. Interindividual variability of thioridazine metabolism in human liver microsomes. Human liver microsomes (0.5 mg protein/ml) were incubated in a 20 mM Tris/HCl buffer (pH = 7.4) with thioridazine (25 μM) and NADPH (1 mM) for 25 min. Each bar represents the mean value $\pm$ S.D. of five determinations from independent incubations.

TABLE 1

A correlation (r value) between the rate of thioridazine metabolism and the velocity of P450-specific reactions in human liver microsomes

Each pair of data was compared by a simple linear regression analysis using the statistical program Prism 2.01 (GraphPad Software, Inc.). Statistical significance was indicated with ** $p < 0.01$ and * $p < 0.05$; N.S. ($p > 0.05$) is statistically not significant.

	Phenacetin *O*-Deethylation (CYP1A2)	Coumarin 7-Hydroxylation (CYP2A6)	*S*-Mephenytoin *N*-Demethylation (CYP2B6)	Diclofenac 4′-Hydroxylation (CYP2C9)	*S*-Mephenytoin 4′-Hydroxylation (CYP2C19)	Bufuralol 1′-Hydroxylation (CYP2D6)	Chlorzoxazone 6-Hydroxylation (CYP2E1)	Testosterone 6β-Hydroxylation (CYP3A4)
Thioridazine mono-2-sulfoxidation	−0.21$^{N.S.}$	0.58$^{N.S.}$	−0.50$^{N.S.}$	0.20$^{N.S.}$	−0.52$^{N.S.}$	**0.74***	0.47$^{N.S.}$	**0.71***
Thioridazine di-2-sulfoxidation	−0.37$^{N.S.}$	−0.06$^{N.S.}$	−0.90$^{N.S.}$	0.05$^{N.S.}$	−0.25$^{N.S.}$	**0.73***	0.18$^{N.S.}$	−0.57$^{N.S.}$
Thioridazine 5-sulfoxidation	**0.77***	0.08$^{N.S.}$	0.09$^{N.S.}$	−0.01$^{N.S.}$	0.24$^{N.S.}$	−0.37$^{N.S.}$	0.08$^{N.S.}$	**0.71***
Thioridazine *N*-demethylation	**0.72***	0.35$^{N.S.}$	0.70$^{N.S.}$	−0.01$^{N.S.}$	0.16$^{N.S.}$	0.25$^{N.S.}$	0.16$^{N.S.}$	**0.84****

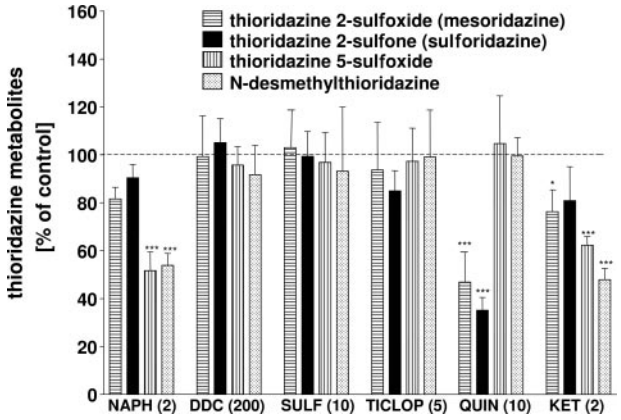


FIG. 4. Effect of P450-selective inhibitors on the rate of thioridazine metabolism in pooled human liver microsomes (HK23, HK25, HK37, HG03, HG43, HG56, HG89, and HG93). Microsomes were incubated with 25 μM thioridazine in the absence (control) or presence of P450-specific inhibitors: 2 μM α-naphthoflavone (NAPH), 200 μM DDC, 10 μM sulfaphenazole (SULF), 5 μM ticlopidine (TICLOP), 10 μM quinidine (QUIN), or 2 μM ketoconazole (KET). Absolute control values were 56.9 ± 13.7 pmol of mesoridazine/mg of protein/min, 4.8 ± 0.8 pmol of sulforidazine/mg of protein/min, 38.7 ± 2.3 pmol of thioridazine 5-sulfoxide/mg of protein/min and 16.0 ± 2.3 pmol of *N*-desmethylthioridazine/mg of protein/min. Mean values ± S.D. ($n = 5$) are presented. Statistical significance was assessed using Student's *t* test and indicated with ***, $p < 0.001$; and *, $p < 0.05$. For further explanation, see Fig. 3.

O-deethylase (CYP1A2) and testosterone 6β-hydroxylase (CYP3A4) activities, whereas the production of thioridazine mono-2-sulfoxide (mesoridazine) and thioridazine di-2-sulfoxide (sulforidazine) was correlated well with bufuralol 1′-hydroxylase activity (CYP2D6). Moreover, thioridazine mono-2-sulfoxidation also correlated significantly with testosterone 6β-hydroxylase activity (CYP3A4).

No correlation was observed between the production of thioridazine metabolites and the rates of coumarin 7-hydroxylation (CYP2A6), *S*-mephenytoin *N*-demethylation (CYP2B6), diclofenac 4′-hydroxylation (CYP2C9), *S*-mephenytoin 4′-hydroxylation (CYP2C19), and chlorzoxazone 6-hydroxylation (CYP2E1).

Inhibition of Thioridazine Metabolism by P450-Selective Inhibitors. α-Naphthoflavone (a CYP1A2 inhibitor) and ketoconazole (a CYP3A4 inhibitor) significantly decreased the rate of thioridazine 5-sulfoxidation (up to 52 and 62% of the control value, respectively) and thioridazine *N*-demethylation (up to 54 and 48% of the control value, respectively) (Fig. 4). On the other hand, quinidine (a CYP2D6 inhibitor) exerted a strong inhibitory effect on the rate of thioridazine mono-2- and di-2-sulfoxidation (up to 48 and 35% of the control value, respectively) (Fig. 4). Moreover, ketoconazole significantly reduced the rate of thioridazine mono-2-sulfoxidation (up to 76% of the control value). DDC (a CYP2A6 + CYP2E1 inhibitor), sulfaphenazole (a CYP2C9 inhibitor), and ticlopidine (a CYP2C19 inhibitor) had no inhibitory effect on the rate of thioridazine metabolism.

Study with cDNA-Expressed Human P450s. The Lineweaver-Burk analysis of the thioridazine metabolism in cDNA-expressed human P450s is presented in Table 2. The obtained kinetic parameters showed distinct inter-isoform differences, which was consistent with the multienzyme Eadie-Hofstee plots derived from liver microsomes (Fig. 2, A–D). The highest intrinsic clearance (V_{max}/K_m) was found for CYP1A2 and CYP3A4 in the case of 5-sulfoxidation and *N*-demethylation, and for CYP2D6 in the case of mono-2- and di-2-sulfoxidation.

The ability of cDNA-expressed human P450s to metabolize thioridazine at its low, therapeutic concentration (10 μM) is shown in Fig. 5, A to D. According to the calculated intrinsic clearance values, the preference of P450 isoforms for catalyzing thioridazine metabolism was as follows (pmol of product/pmol of P450 isoform/min): 2D6 > 2C19 > 1A2 ≈ 2B6 > 3A4 > 2C9 for mono-2-sulfoxidation; 2D6 > 2C19 > 1A2 > 3A4 for di-2-sulfoxidation; 1A2 > 3A4 > 2E1 ≈ 2A6 > 2B6 > 2D6 > 2C9 > 2C19 for 5-sulfoxidation; and 1A2 > 2C19 > 3A4 > 2D6 for *N*-demethylation. CYP2A6 and CYP2AE1 did not produce mesoridazine at a measurable amount, whereas sulforidazine and *N*-desmethylthioridazine were not formed by CYP2A6, CYP2B6, CYP2C9, and CYP2E1.

Quantitative Estimation of the Contribution of P450 Enzymes to the Particular Metabolic Pathways of Thioridazine. We roughly

TABLE 2

Kinetic parameters of thioridazine metabolism in cDNA-expressed human P450s (Supersomes)

P450	Thioridazine Mono-2-sulfoxidation			Thioridazine Di-2-sulfoxidation			Thioridazine 5-Sulfoxidation			Thioridazine *N*-Demethylation		
	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m
	μM	*pmol/mg protein/min*		*μM*	*pmol/mg protein/min*		*μM*	*pmol/mg protein/min*		*μM*	*pmol/mg protein/min*	
CYP1A2	160	1.786	0.0112	16	0.020	0.0012	80	7.519	**0.0940**	36	0.403	**0.0112**
CYP2A6	323	0.485	0.0015	48	0.004	0.0001	34	0.879	0.0258	200	0.200	0.0010
CYP2B6	67	0.605	0.0090	55	0.006	0.0001	71	1.412	0.0199	55	0.124	0.0022
CYP2C9	45	0.221	0.0049	91	0.009	0.0001	114	1.250	0.0110	20	0.057	0.0028
CYP2C19	47	6.289	0.1338	26	0.141	0.0054	800	6.993	0.0087	400	2.128	0.0053
CYP2D6	62	16.667	**0.2688**	10	0.468	**0.0468**	385	5.263	0.0137	67	0.233	0.0035
CYP2E1	333	0.527	0.0016	38	0.004	0.0001	50	1.160	0.0232	83	0.109	0.0013
CYP3A4	42	0.302	0.0072	20	0.009	0.0004	28	1.193	**0.0426**	21	0.148	**0.0070**

The highest intrinsic clearances are shown in bold.

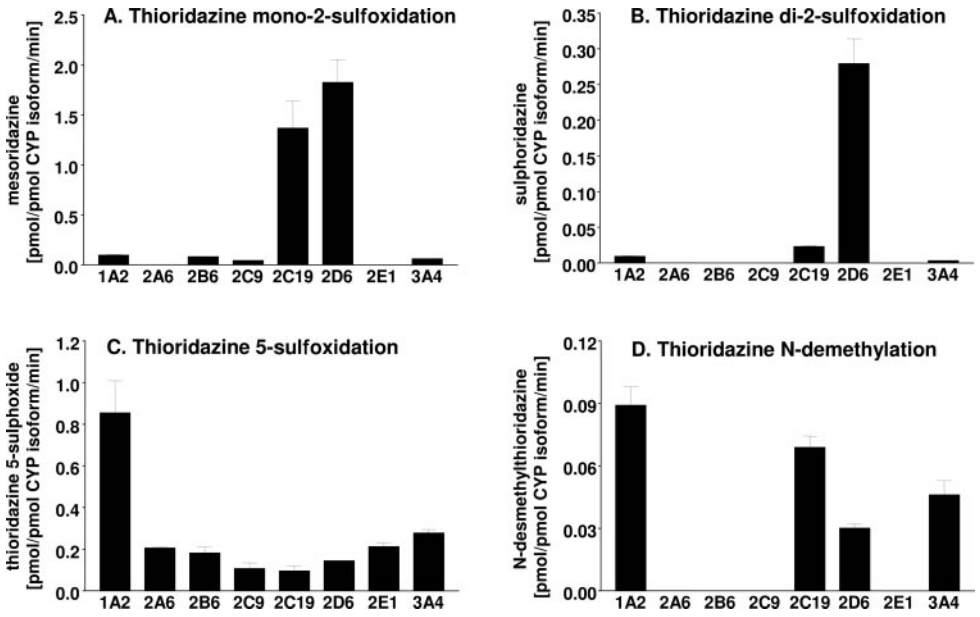


FIG. 5. Biotransformation of thioridazine via mono-2-sulfoxidation (A), di-2-sulfoxidation (B), 5-sulfoxidation (C), and *N*-demethylation (D) by the cDNA-expressed human P450s (Supersomes). Thioridazine (10 μM) was incubated with Supersomes (100 pmol P450/ml) and NADPH (1 mM) for 25 min. Each bar represents the mean value $\pm$ S.D. of three determinations from independent incubations.

estimated the contribution of the P450 enzymes studied here to thioridazine mono-2-, di-2-, and 5-sulfoxidation, and *N*-demethylation in liver microsomes on the basis of the rate of those reactions in the Supersomes and the contribution of each isoform to the total P450 content in the human liver. Calculations performed at a low (therapeutic) concentration of thioridazine (10 μM) indicate that CYP2D6 is the main isoform responsible for mono-2- and di-2-sulfoxidation (48.6 and 63.6%, respectively), whereas CYP1A2 and CYP3A4 are the key enzymes that catalyze 5-sulfoxidation (46.5 and 34.3%, respectively) and *N*-demethylation (44.1 and 51.6%, respectively) of thioridazine in the human liver (Table 3). CYP3A4 contributes to a lesser degree to thioridazine mono-2-sulfoxidation (21.7%) (Table 3). The results obtained at 10 μM and 100 μM (data not shown) thioridazine were similar.

In the liver, the amount of a metabolite formed by an individual P450 enzyme depends on both its catalytic activity with respect to the product formation and its relative contribution to the total P450 content. Therefore, although the intrinsic clearance found for CYP2C19 was higher than that for CYP3A4 regarding mono-2-sulfoxidation (Table 2), the calculated contribution of CYP3A4 to this reaction was considerably greater than that of CYP2C19 (Table 3), as a result of their relative amounts in the total liver P450 (30% and 1%, respectively).

Discussion

The results presented above indicate a major contribution of CYP1A2 and CYP3A4 to thioridazine 5-sulfoxidation and *N*-demethylation, and of CYP2D6 to its mono-2- and di-2-sulfoxidation. It is also worth stressing that CYP3A4 contributes to mono-2-sulfoxidation of thioridazine. The above final conclusion is based on our consistent results of the Eadie-Hofstee analysis (nonlinear plots suggesting multiple-enzyme catalysis), correlation and inhibition studies, and demonstration of the ability of cDNA-expressed P450s to metabolize thioridazine. It should be mentioned that the latter results (based on the average values of P450s in the liver) are theoretical, since actual results would depend on the interindividual variability of P450s and the diverse contribution of individual P450s to the total content of P450 protein in the liver.

The obtained results are similar to our recent data (Wójcikowski et al., 2003, 2004) showing the main contribution of CYP1A2 and CYP3A4 to

TABLE 3

Estimation of the contribution of P450 isoforms to the particular metabolic pathways of thioridazine (10 μM) on the basis of the rates of these reaction in Supersomes and average P450 contents in the liver

P450	Relative Contribution of the Isoform to the total P450 Contents in Liver Microsomes (Fraction)*	Relative Contribution of the Isoform to Thioridazine Metabolism in Liver Microsomes (Percentage)†			
		Thioridazine Mono-2-sulfoxidation	Thioridazine Di-2-sulfoxidation	Thioridazine 5-Sulfoxidation	Thioridazine *N*-Demethylation
CYP1A2	0.127[a]	10.8	16.7	46.5	44.1
CYP2A6	0,040[a]	N.D.	N.D.	3.5	N.D.
CYP2B6	0.002[a]	0.2	N.D.	0.2	N.D.
CYP2C9	0.180[b]	6.6	N.D.	8.2	N.D.
CYP2C19	0.010[b]	12.1	6.1	0.4	2.7
CYP2D6	0.015[a]	48.6	63.6	0.9	1.6
CYP2E1	0.066[a]	N.D.	N.D.	6.0	N.D.
CYP3A4	0.288[a]	21.7	13.6	34.3	56.1

N.D., not detected.

* Data according to [a] Shimada et al. (1994) and [b] Lewis (2001).

† Relative contribution of P450s to the particular metabolic pathways of thioridazine was calculated as percentage of the sum of predicted velocities in liver microsomes. The predicted velocity in liver microsomes was calculated by multiplying the velocity in Supersomes (see Fig. 5) by the relative contribution of isoform to the total P450 content in liver microsomes. For details, see Wójcikowski et al. (2003, 2004).

the 5-sulfoxidation (in the thiazine ring) of the aliphatic-type phenothiazine neuroleptic promazine (31 and 39%, respectively) and the piperazine-type neuroleptic perazine (32 and 30%, respectively). As for promazine and perazine, the 5-sulfoxidation of thioridazine (also in the thiazine ring) was mediated by CYP1A2 (46%) and CYP3A4 (34%). However, some interdrug differences were observed in the catalysis of side chain *N*-demethylation. The contribution of CYP1A2 and CYP2C19 to promazine *N*-demethylation was similar (35 and 32%, respectively), whereas CYP2C19 was the main isoenzyme catalyzing perazine *N*-demethylation (68%). On the contrary, CYP2C19 contributed only marginally to thioridazine *N*-demethylation (4%). This metabolic pathway of thioridazine was mediated by CYP1A2 (44%) and CYP3A4 (52%).

The above discrepancy may stem from different chemical structures of the side chains of the phenothiazines studied (thioridazine-piperidine side chain, promazine-aliphatic side chain, perazine-piperazine side chain), which influence their access to and the interaction with catalytic sites of cytochrome P450. The minor contribution of CYP2C19 to thioridazine *N*-demethylation in a side chain might be due to strict structural requirements of the enzyme for its substrates and to limited space around the nitrogen in the side chain (compared with promazine and perazine), which hinders oxidation of the chemically vulnerable position by CYP2C19.

The results obtained in our experiment agree with clinical studies demonstrating that CYP2D6 is the key enzyme catalyzing thioridazine 2-sulfoxidation (Meyer et al., 1990; Llerena et al., 2000, 2001; Berecz et al., 2003) and that CYP1A2, in addition to CYP2D6, is involved in the metabolism of thioridazine (Berecz et al., 2003). However, our calculated data showed that besides CYP2D6 (49%), CYP3A4 (22%) was substantially engaged in mono-2-sulfoxidation of thioridazine. Thus, considering the higher contribution of CYP2D6 to di-2-sulfoxidation (64%) than to mono-2-sulfoxidation (49%) of thioridazine, it seems that the sulforidazine/mesoridazine ratio might be an additional and more specific marker of CYP2D6 activity than the mesoridazine/thioridazine ratio, proposed by Llerena et al. (2000). The above suggestion is supported by the studies of von Bahr et al. (1991), which showed that the plasma concentration of sulforidazine was influenced by the debrisoquine hydroxylation phenotype more than was the mesoridazine level in humans. Moreover, considering an easily detectable concentration of sulforidazine in human plasma (although one half to one quarter of the concentration of mesoridazine) (Cohen et al., 1989; Meyer et al., 1990), the simultaneous measurement of both metabolites in plasma and determination of the two markers of CYP2D6 activity is possible. It is also important to note that in light of our results, the CYP2D6 activity indicator expressed as the sulforidazine/mesoridazine ratio is independent (in contrast to the mesoridazine/thioridazine ratio) of the interindividual differences in expression of CYP3A4, which may vary between patients up to 60 times (Shimada et al., 1994; Hustert et al., 2001).

Our data concerning the contribution of particular cytochrome P450 isoenzymes to the metabolism of thioridazine may have significant implications for the prediction of drug-drug interactions. Thioridazine is administered to patients for months or years, very often in combination with antidepressant, antimanic, or antianxiety drugs, which engage the same P450 enzymes for their metabolism. Considering the serious side effects of thioridazine and some of its metabolites, metabolic interaction between thioridazine and other psychotropics may be of clinical importance. Metabolic interactions of this type between thioridazine and antidepressant drugs have been found in rats (Daniel et al., 1999, 2000), and their serious consequences were observed in humans (Heiman, 1977).

In summary, the results of the present study show that 1) different structures of phenothiazine neuroleptics (mainly the structure of a side chain) influence their interactions with catalytic sites of cytochrome P450; 2) CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulfoxidation and *N*-demethylation, whereas CYP2D6 is the basic enzyme that catalyzes mono-2- and di-2-sulfoxidation of thioridazine in human liver; 3) besides CYP2D6, CYP3A4 significantly contributes to thioridazine mono-2-sulfoxidation; 4) the relative contribution of CYP2C19 to thioridazine *N*-demethylation is marginal; and 5) the sulforidazine/mesoridazine ratio might be an additional and more specific marker of CYP2D6 activity than the mesoridazine/thioridazine ratio.

References

Axelsson R (1977) On the serum concentrations and antipsychotic effects of thioridazine, thioridazine side-chain sulfoxide and thioridazine side-chain sulfone in chronic psychotic patients. *Curr Ther Res Clin Exp* **21:**587–605.

Berecz R, De La Rubia A, Dorado P, Fernandez-Salguero P, Dahl M-L, and Llerena A (2003) Thioridazine steady-state plasma concentrations are influenced by tobacco smoking and CYP2D6, but not by the CYP2C9 genotype. *Eur J Clin Pharmacol* **59:**45–50.

Bylund DB (1981) Interactions of neuroleptic metabolites with dopaminergic, alpha adrenergic and muscarinic cholinergic receptors. *J Pharmacol Exp Ther* **217:**81–86.

Cohen BM, Lipiński JF, and Waternaux C (1989) A fixed dose study of the plasma concentration and clinical effects of thioridazine and its major metabolites. *Psychopharmacology* **97:**481–488.

Daniel WA, Syrek M, Haduch A, and Wójcikowski J (1999) The influence of selective serotonin reuptake inhibitors (SSRIs) on the pharmacokinetics of thioridazine and its metabolites: in vivo and in vitro studies. *Exp Toxicol Pathol* **51:**309–314.

Daniel WA, Syrek M, Haduch A, and Wójcikowski J (2000) Pharmacokinetics and metabolism of thioridazine during co-administration of tricyclic antidepressants. *Br J Pharmacol* **131:** 287–295.

Daniel WA, Syrek M, Mach A, Wójcikowski J, and Boksa J (1997) Pharmacokinetics of thioridazine and its metabolites in blood plasma and the brain of rats after acute and chronic treatment. *Pol J Pharmacol* **49:**439–452.

Gottschalk LA, Dinovo E, Biener R, and Nandi BR (1978) Plasma concentrations of thioridazine metabolites and ECG abnormalities. *J Pharm Sci* **67:**155–157.

Hale PW Jr and Poklis A (1986) Cardiotoxicity of thioridazine and two stereoisomeric forms of thioridazine 5-sulfoxide in the isolated perfused rat heart. *Toxicol Appl Pharmacol* **86:**44–55.

Heiman EM (1977) Cardiac toxicity with thioridazine-tricyclic antidepressant combination. *J Nerv Ment Dis* **165:**139–143.

Hustert E, Zibat A, Prescan-Siedel E, Eiselt R, Mueller R, Fub C, Brehm I, Brinkmann U, Eichelbaum M, Wojnowski L, et al. (2001) Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. *Drug Metab Dispos* **29:**1454–1459.

Hyttel J, Larsen JJ, Christensen AV, and Amt J (1985) Receptor binding profiles of neuroleptics, in *Dyskinesia—Research and Treatment* (Casey DE, Chase TN, Christensen AV, and Gerlach J eds) pp 9–18, Springer, Berlin.

Lewis DFV (2001) Substrate selectivity and metabolism, in *Cytochromes P450. Structures and Function,* pp 76–117, Taylor and Francis, London.

Lin G, Hawes EM, Mckay G, Korchinski ED, and Midha KK (1993) Metabolism of piperidine-type phenothiazine antipsychotic agents. IV. Thioridazine in dog, man and rat. *Xenobiotica* **23:**1095–1074.

Llerena A, Berecz R, De La Rubia A, and Dorado P (2002) QTc interval lengthening is related to CYP2D6 hydroxylation capacity and plasma concentration of thioridazine in patients. *J Psychopharmacol* **16:**361–364.

Llerena A, Berecz R, De La Rubia A, Fernandez-Salguero P, and Dorado P (2001) Effect of thioridazine dosage on the debrisoquine hydroxylation phenotype in psychiatric patients with different CYP2D6 genotypes. *Ther Drug Monit* **23:**616–620.

Llerena A, Berecz R, De La Rubia A, Norberto MJ, and Benitez J (2000) Use of the mesoridazine/thioridazine ratio as a marker for CYP2D6 enzyme activity. *Ther Drug Monit* **22:**397–401.

Meyer J, Woggon B, Baumann P, and Meyer UA (1990) Clinical implications of slow sulphoxidation of thioridazine in poor metabolizer of the debrisoquine type. *Eur J Clin Pharmacol* **39:**613–614.

Papadopoulos AS, Crammer JL, and Cowan DA (1985) Phenolic metabolites of thioridazine in man. *Xenobiotica* **15:**309–316.

Richelson E and Nelson A (1984) Antagonism by neuroleptics of neurotransmitter receptors of normal human brain in vitro. *Eur J Pharmacol* **103:**197–204.

Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270:**414–423.

Svendsen CN and Bird ED (1986) HPLC with electrochemical detection to measure chlorpromazine, thioridazine and metabolites in human brain. *Psychopharmacology* **90:**316–321.

von Bahr C, Movin G, Nordin C, Liden A, Hammarlund-Udenaes M, Hedberg A, Ring H, and Sjögvist F (1991) Plasma levels of thioridazine and metabolites are influenced by the debrisoquin hydroxylation phenotype. *Clin Pharmacol Ther* **49:**234–240.

Wójcikowski J, Pichard-Garcia L, Maurel P, and Daniel WA (2003) Contribution of human cytochrome P-450 isoforms to the metabolism of the simplest phenothiazine neuroleptic promazine. *Br J Pharmacol* **138:**1465–1474.

Wójcikowski J, Pichard-Garcia L, Maurel P, and Daniel WA (2004) The metabolism of the piperazine-type phenothiazine neuroleptic perazine by the human cytochrome P-450 isoenzymes. *Eur Neuropsychopharmacol* **14:**199–208.

Address correspondence to: Dr. Jacek Wójcikowski, Polish Academy of Sciences, Institute of Pharmacology, Smętna 12, 31-343 Kraków, Poland. E-mail: wojcikow@if-pan.krakow.pl