Biochemical Pharmacology 80 (2010) 1252–1259

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Main contribution of the cytochrome P450 isoenzyme 1A2 (CYP1A2) to N-demethylation and 5-sulfoxidation of the phenothiazine neuroleptic chlorpromazine in human liver—A comparison with other phenothiazines

Jacek Wójcikowski *, Jan Boksa, Władysława A. Daniel

Polish Academy of Sciences, Institute of Pharmacology, Smętna 12, 31-343 Kraków, Poland

ARTICLE INFO

Article history:
Received 20 April 2010
Accepted 28 June 2010

Keywords:
Chlorpromazine
Metabolism
Human CYP
Microsomes
Supersomes
Specific inhibitors

ABSTRACT

The aim of the present study was to identify cytochrome P450 (CYP) isoenzymes involved in the 5-sulfoxidation, mono-N-demethylation and di-N-demethylation of the aliphatic-type phenothiazine neuroleptic chlorpromazine in human liver. Experiments were performed *in vitro* using cDNA-expressed human CYP isoforms (Supersomes 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4), liver microsomes from different donors and CYP-selective inhibitors. The obtained results indicate that CYP1A2 is the only CYP isoform that catalyzes the mono-N-demethylation and di-N-demethylation of chlorpromazine (100%) and is the main isoform responsible for chlorpromazine 5-sulfoxidation (64%) at a therapeutic concentration of the drug (10 μM). CYP3A4 contributes to a lesser degree to chlorpromazine 5-sulfoxidation (34%). The role of CYP2B6, CYP2C19 and CYP2D6 in catalyzing of the latter reaction is negligible (0.1–2%). Similar results were obtained at a higher, non-therapeutic concentration of the drug (100 μM); however, the contribution of CYP1A2 to chlorpromazine mono-N-demethylation was noticeably lower (75%), mostly in favour of CYP2C19 and CYP3A4 (about 12% each). The obtained results indicate that the catalysis of chlorpromazine N-demethylation and 5-sulfoxidation in humans exhibits a stricter CYP1A2 preference compared to the previously tested phenothiazines (promazine, perazine, and thioridazine). Hence pharmacokinetic interactions involving chlorpromazine and CYP1A2 substrates and inhibitors are likely to occur. Considering strong dopaminergic D_2, noradrenergic α_1 and cholinergic M_1 receptor blocking properties of chlorpromazine and some of its metabolites, as well as their serious side effects, the obtained results may be of pharmacological and clinical importance.

1. Introduction

Chlorpromazine belongs to the group of phenothiazine neuroleptics of an aliphatic type. It is still the most widely used phenothiazine to treat schizophrenic patients, especially in the developing world [1]. Chlorpromazine is a strong antagonist of the dopaminergic D_2 receptor which is responsible for its antipsychotic effect and is a blocker of adrenergic α_1 and muccarinic M_1 receptors which might be associated with some side effects of the drug, such as hypotension, sedation and an anticholinergic symptoms. Furthermore, it is a weak antagonist of histamine H_1 and serotonergic 5-HT_2 receptors. Apart from α_1-receptor blocade, antihistaminic effect is also considered to be connected with the sedative side effect of chlorpromazine [2–4].

During phase I of metabolism, chlorpromazine undergoes *S*-oxidation in the thiazine ring in position 5, mono-N-demethylation and di-N-demethylation in a side chain, as well as aromatic hydroxylation (mainly in position 7) and N-oxidation [5–8] (Fig. 1). Receptor binding studies have shown that 7-hydroxychlorpromazine and mono-N-desmethylchlorpromazine are the most active metabolites of the drug. These metabolites possess about 50% of the parent compound activity in blocking D_2 and α_1 receptors. On the other hand, high levels of chlorpromazine 5-sulfoxide appear to have a negative effect on therapeutic response in schizophrenic patients. All these metabolites are much weaker antagonists of the M_1 receptor than is the parent compound [9–11]. Hence the biotransformation of chlorpromazine leads to the formation of different derivatives, most of which display biological activity and may contribute to the therapeutic and/or side effects of the parent drug.

It is still unclear which enzymes are responsible for particular metabolic steps of chlorpromazine. Using human microsomes and cDNA-expressed human CYP isoforms, Yoshii et al. [12] showed that chlorpromazine 7-hydroxylation was catalyzed mainly by CYP2D6, and to a lesser extent by CYP1A2. On the other hand, using human liver microsomes and a high concentration of the neuroleptic (200 μM), Cashman et al. [13] found that the rate of chlorpromazine 5-sulfoxidation was significantly reduced by

* Corresponding author. Tel.: +48 12 6374022; fax: +48 12 6374500.
E-mail address: wojcikow@if-pan.krakow.pl (J. Wójcikowski).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.06.045

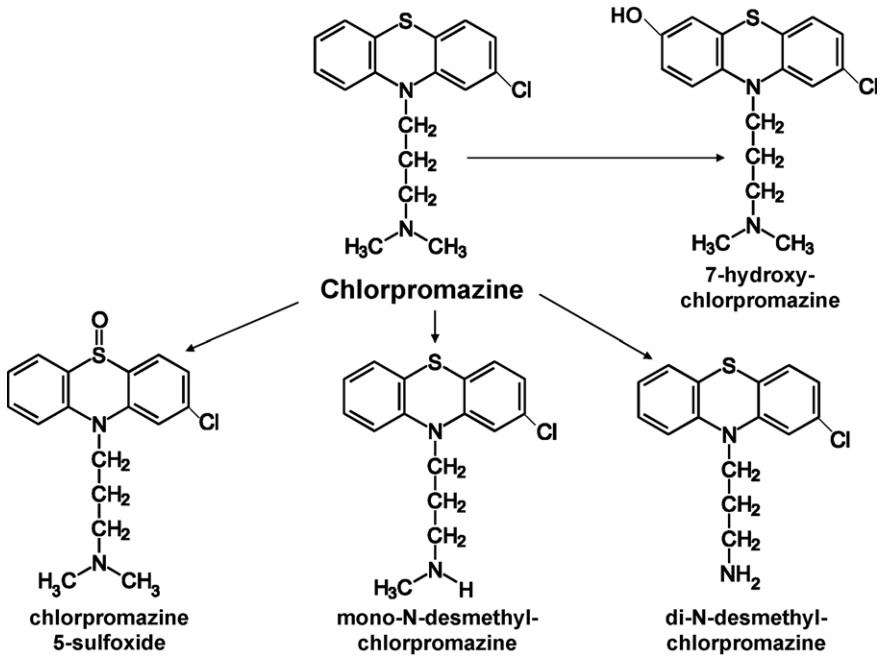


Fig. 1. The main metabolic pathways of chlorpromazine.

troleandomycin (a CYP3A4 inhibitor). Clinical studies showed that CYP1A2 polymorphism may be involved in the risk of developing a prolonged QT-interval in patients who were treated with higher doses of chlorpromazine [14]. It was also shown that chlorpromazine clearance in schizophrenic patients was enhanced by tobacco smoking (CYP1A2 induction) [15]. Moreover, studies conducted on rat liver microsomes demonstrated that chlorpromazine inhibited imipramine N-demethylation, a reaction which is catalyzed mainly by CYP1A2 and CYP3A4 in humans [16,17]. The above data suggest that besides CYP3A4, CYP1A2 may also be engaged in the metabolism of chlorpromazine.

Thus, there are no complete data on the enzymatic catalysis of chlorpromazine metabolism in humans. The catalysis of chlorpromazine mono-N-demethylation and di-N-demethylation has not been studied in humans so far. On the other hand, studies on the involvement of CYP isoforms in the metabolism of chlorpromazine 5-sulfoxidation were carried out at a high, non-pharmacological substrate concentration and in the presence of a CYP3A4 inhibitor only [13]. Nonetheless, a possible effect of other CYP inhibitors on chlorpromazine 5-sulfoxidation was not studied in the latter study. In particular, there have not been conducted so far any experiments using a low, therapeutic concentration of chlorpromazine or a full set of human cDNA-expressed CYP enzymes. Therefore the aim of the present study was to qualitatively and quantitatively estimate the contribution of human CYP isoforms to chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-N-demethylation at different drug concentrations (low – e.g., therapeutic and high). To achieve this aim we used a full set of cDNA-expressed CYP enzymes, and the obtained results were confirmed with complementary *in vitro* models (inhibition and correlation studies in liver microsomes).

2. Materials and methods

2.1. Drugs and chemicals

Chlorpromazine hydrochloride was obtained from Jelfa (Jelenia Góra, Poland). Mono-N-desmethylthioridazine and di-N-desmethylthioridazine were donated by Prof. M.H. Bickel (University of Bern, Switzerland). Chlorpromazine 5-sulfoxide was synthesized in our laboratory as described below. Furafylline, DDC (diethyldithiocarbamic acid), ThioTEPA (N,N′,N″-triethylenethiophosphoranide), sulfaphenazol, (+)-N-3-benzyl-nirvanol, quinidine, ketoconazole and NADPH were purchased from Sigma (St. Louis, USA). All the organic solvents with HPLC purity were supplied by Merck (Darmstadt, Germany).

2.2. Synthesis of chlorpromazine 5-sulfoxide

The synthesis procedure was based on the method of Yeung et al. [18]. Chlorpromazine hydrochloride, 0.459 g, and potassium nitrite, 0.5 g, were dissolved in 30 ml of distilled water containing 0.7 ml of hydrochloric acid. After 30 min, the mixture was alkalized with 5 ml of a 25% aqueous ammonia. The solution was extracted three times with 50 ml of chloroform and evaporated. The residue (yellow oil) was dissolved in the mixture of acetone (3 ml) and hydrochloric acid (1 ml). The solvent was decanted and the residue was then crystallized from a solution of ethanol (5 ml) and diethyl ether (5 ml). The obtained substance (in the form of a hydrochloride, 0.4 g) had a m.p. of 125–127 °C and was chromatographically homogenous.

2.3. Human liver microsomes

Human liver microsome preparations (HG03, HG24, HG56, HG88, HG93, HG95, HG103, HH18, HH35, HH40 and HK25) were obtained from Gentest Co. (Woburn, MA, USA).

Liver microsomes from patients HG95, HG103 and HH40 were used for optimizing the conditions of chlorpromazine metabolism. On the basis of the obtained results, chlorpromazine metabolism in liver microsomes was studied with respect to the linear dependence of product formation on the time and concentrations of protein and the substrate. Microsomal protein, 1 mg, was resuspended in 1000 μl of 0.15 M phosphate buffer (pH = 7.4). To determine enzyme kinetic parameters, the chlorpromazine concentrations used ranged from 10 to 200 μM. For studies of chlorpromazine metabolism in individual patients 10 μM chlorpromazine was used. For inhibition studies, 10 μM chlorpromazine was incubated with the selective CYP inhibitors: 10 μM furafylline (a CYP1A2 inhibitor), 200 μM DDC (a CYP2A6 + CYP2E1

inhibitor), 10 μM ThioTEPA (a CYP2B6 inhibitor), 10 μM sulfaphenazole (a CYP2C9 inhibitor), 2 μM (+)-N-3-benzyl-nirvanol (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. Chlorpromazine and its metabolites were analyzed by the HPLC method as described below.

2.4. Correlation analysis of the data

The rates of chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-N-demethylation in liver microsomes from 12 donors were correlated with the rates of CYP-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 pair of the data was compared by a simple linear regression analysis using the statistical programme Prism 2.01. Monooxygenase activities for each liver microsomal preparation (donor) were determined and provided by Gentest Co. (Woburn, MA, USA).

2.5. cDNA-expressed human CYP isoforms

Microsomes from baculovirus-infected insect cells expressing CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 (Supersomes) were obtained from Gentest Co. (Woburn, MA, USA). Chlorpromazine metabolism was studied under experimental conditions similar to those described for liver microsomes, with 10, 25, 50, 100 and 200 μM chlorpromazine, except for the fact that the final concentration of CYPs was 100 pmol/ml. Chlorpromazine and its metabolites were analyzed by HPLC as described below.

2.6. Determination of chlorpromazine and its metabolites in the incubation medium

Chlorpromazine and its metabolites were quantified using the slightly modified HPLC method of Wójcikowski et al. [22]. Briefly, after incubation, the samples were centrifuged for 10 min at 2000 × *g*. The water phase containing chlorpromazine and its metabolites was extracted (pH = 12) with diethyl ether and dichloromethane (3;1, v/v). 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 chlorpromazine and its metabolites (chlorpromazine 5-sulfoxide, mono-N-desmethylchlorpromazine and di-N-desmethylchlorpromazine) were assayed using a LaChrom (Merck-Hitachi) HPLC system with UV detection. The analytical column (Econosphere C18, 5 μm, 4.6 × 250 mm) was purchased from Alltech (Carnforth, England). 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 of 50:50. The flow rate was 0.6 ml/min, the column temperature was 30 °C. The absorbance of chlorpromazine and its metabolites was measured at a wavelength of 254 nm. The compounds were eluted in the following order: chlorpromazine 5-sulfoxide (10.31 min), di-N-desmethylchlorpromazine (13.31 min), chlorpromazine (15.41 min) and mono-N-desmethylchlorpromazine (16.81 min).

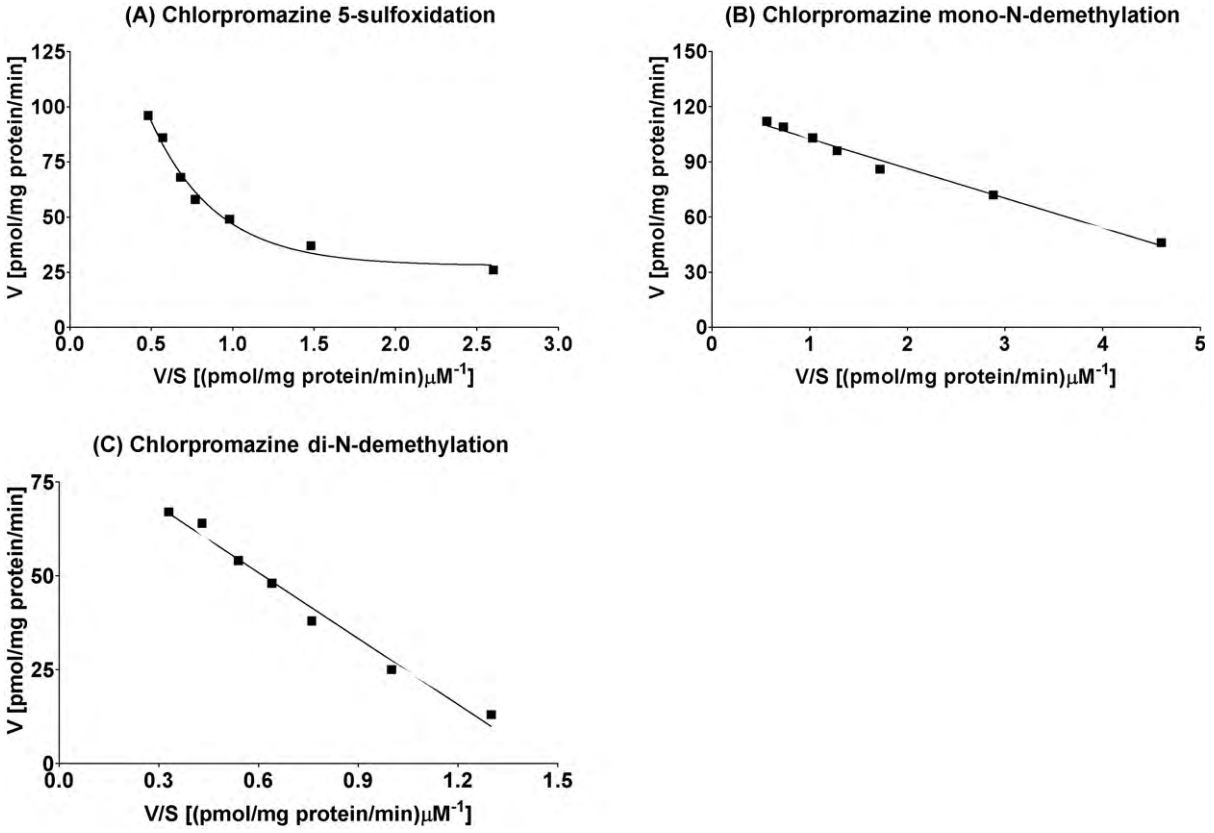


Fig. 2. The Eadie–Hofstee plots for chlorpromazine 5-sulfoxidation (A), mono-N-demethylation (B) and di-N-demethylation (C) in human liver microsomes. The human liver microsomes of patient HK95 (1 mg of protein/ml) were incubated in a 0.15 M phosphate buffer (pH = 7.4) with chlorpromazine (5–200 μM) and NADPH (1 mM) for 25 min. Similar results were obtained for the microsomes of patients HG103 and HH40.

The sensitivity of the HPLC method allowed us to quantify chlorpromazine as low as 0.001 nmol/ml, chlorpromazine 5-sulfoxide as low as 0.004 nmo/ml, di-N-desmethylchlorpromazine as low as 0.002 nmo/ml and mono-N-desmethylchlorpromazine as low as 0.004 nmo/ml of the microsomal suspension. The accuracy of the method amounted to 2%. The inter- and intra-assay coefficients of variance were below 2% and 3%, respectively.

3. Results

3.1. Chlorpromazine metabolism in human liver microsomes

Fig. 2A–C shows representative Eadie–Hofstee plots for the process of chlorpromazine oxidation in liver microsomes from patient HG95. These plots suggest that only one enzyme is responsible for the biotransformation of chlorpromazine via mono-N-demethylation and di-N-demethylation (linear plots), while multiple enzymes are engaged in chlorpromazine 5-sulfoxidation (a non-linear plot). Similar results were obtained for liver microsomes from patients HG103 and HH40 (data not shown).

3.2. Correlation study

Interindividual variability of the rates of chlorpromazine metabolism ranged from 3.8-fold (di-N-demethylation) to 5.5-fold (5-sulfoxidation) (Fig. 3).

The rates of formation of chlorpromazine metabolites by different preparations of human liver microsomes (Fig. 3) were compared with the monooxygenase activities determined for each liver preparation by the supplier (Gentest Co.). 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 the data. The formation of chlorpromazine 5-sulfoxide, mono-N-desmethylchlorpromazine and di-N-desmethylchlorpromazine strongly correlated with phenacetin *O*-deethylase activity (CYP1A2). Moreover, chlorpromazine 5-sulfoxidation also correlated significantly with testosterone 6β-hydroxylase activity (CYP3A4). No correlation was observed between the production of chlorpromazine metabolites and the rates of coumarin 7-hydroxylation (CYP2A6), *S*-mephenytoin N-demethylation (CYP2B6), diclofenac 4′-hydroxylation (CYP2C9), *S*-mephenytoin 4′-hydroxylation (CYP2C19), bufuralol 1′-hydroxylation (CYP2D6) and chlorzoxazone 6-hydroxylation (CYP2E1).

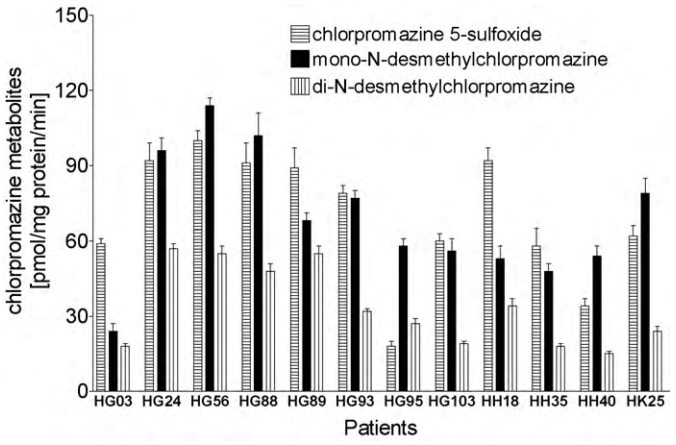


Fig. 3. The interindividual variability of chlorpromazine metabolism in human liver microsomes. Human liver microsomes (1 mg of protein/ml) were incubated in a 0.15 M phosphate buffer (pH = 7.4) with chlorpromazine (10 μM) and NADPH (1 mM) for 25 min. Each bar represents the mean value ± s.d. of five determinations from independent incubations.

Table 1
A correlation (r value) between the rate of chlorpromazine metabolism and the velocity of CYP-specific reactions in human liver microsomes (n = 12).

	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)
Chlorpromazine 5-sulfoxidation	**0.776****	0.447^{ns}	0.517^{ns}	0.121^{ns}	0.371^{ns}	−0.456^{ns}	−0.035^{ns}	**0.581***
Chlorpromazine mono-N-demethylation	**0.889*****	−0.075^{ns}	0.059^{ns}	0.408^{ns}	−0.171^{ns}	0.176^{ns}	−0.112^{ns}	−0.112^{ns}
Chlorpromazine di-N-demethylation	**0.892*****	0.194^{ns}	0.385^{ns}	−0.192^{ns}	0.466^{ns}	−0.307^{ns}	0.035^{ns}	0.182^{ns}

Each pair of data was compared by a simple linear regression analysis using the statistical programme Prism 2.01. $^{ns}(p > 0.05)$ – statistically not significant. Statistical significance was indicated with:
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

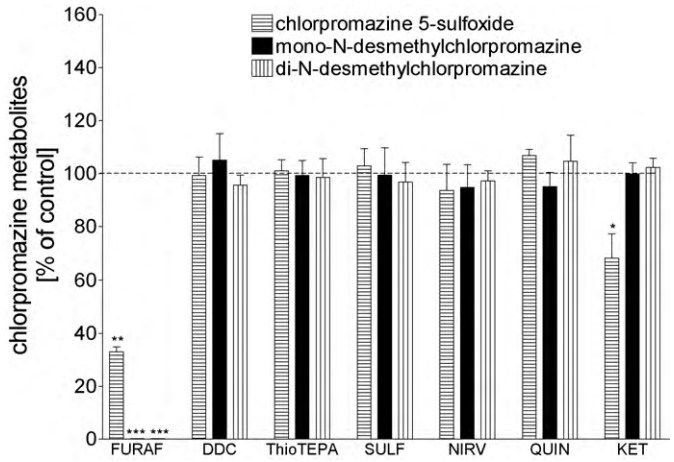


Fig. 4. The effect of CYP-selective inhibitors on the rate of chlorpromazine metabolism in pooled human liver microsomes (HH12, H39, HH41, HH55, HH61, HG08, HG83, HG85, and HG103). The microsomes were incubated with 10 μM chlorpromazine in the absence (control) or presence of CYP-specific inhibitors: 10 μM furafylline (FURAF), 200 μM diethyldithiocarbamic acid (DDC), 10 μM N,N′,N″-triethylenethiophosphoranide (ThioTEPA), 10 μM sulfaphenazole (SULF), 2 μM (+)-N-3-benzyl-nirvanol (NIRV), 10 μM quinidine (QUIN) or 2 μM ketoconazole (KET). The absolute control values were 63.5 ± 4.7 pmol of chlorpromazine 5-sulfoxide/mg protein/min, 59.1 ± 4.3 pmol of mono-N-desmethylchlorpromazine/mg protein/min and 30.7 ± 1.9 pmol of di-N-desmethylchlorpromazine/mg protein/min. The mean values ± s.d. (n = 5) are presented. Statistical significance was assessed using Student's t-test and indicated with $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$. For further explanations see Fig. 3.

3.3. Inhibition of chlorpromazine metabolism by CYP-selective inhibitors

Furafylline (a CYP1A2 inhibitor) and ketoconazole (a CYP3A4 inhibitor) significantly decreased the rate of chlorpromazine 5-sulfoxidation (to 33% and 68% of the control value, respectively) (Fig. 4). On the other hand, furafylline completely inhibited the formation of mono-N-desmethylchlorpromazine and di-N-desmethylchlorpromazine in liver microsomes (Fig. 4). DDC (a CYP2A6 + CYP2E1 inhibitor), ThioTEPA (a CYP2B6 inhibitor), sulfaphenazol (a CYP2C9 inhibitor), (+)-N-3-benzyl-nirvanol (a CYP2C19 inhibitor) and qinidine (a CYP2D6 inhibitor) had no inhibitory effect on the rate of chlorpromazine metabolism (Fig. 4).

3.4. Study with cDNA-expressed human CYP isoforms

The ability of cDNA-expressed human CYP enzymes to metabolize chlorpromazine at its low, therapeutic concentration (10 μM), as well as at a higher, non-therapeutic concentration (100 μM) is shown in Fig. 5A–C. Chlorpromazine 5-sulfoxide was formed by several CYP enzymes at the tested concentrations of chlorpromazine (10 μM and 100 μM), whereat CYP1A2 produced that metabolite in a great amount. Mono-N-desmethylchlorpromazine and di-N-desmethylchlorpromazine were produced exclusively by CYP1A2 at a low neuroleptic concentration (10 μM) only, and mainly by CYP1A2, and – to a lower extent – by CYP2B6, CYP2C19 and CYP3A4 at a higher drug concentration (100 μM).

Kinetic parameters showing chlorpromazine metabolism by the main cDNA-expressed human CYP isoforms which contribute to the metabolism of chlorpromazine, obtained using a non-linear analysis (Program Sigma Plot 8.0; Enzyme Kinetics), are presented in Table 2. The highest intrinsic clearance (V_{max}/K_m) was found for CYP1A2 and CYP3A4 in the case of the 5-sulfoxidation, and for CYP1A2 in the case of the mono-N-demethylation and di-N-demethylation of chlorpromazine (Table 2).

4. Discussion

The results presented above indicate that CYP1A2 is the only CYP isoform that catalyzes the mono-N-demethylation and di-N-demethylation of chlorpromazine, as well as the main enzymes responsible for chlorpromazine 5-sulfoxidation at a therapeutic concentration of the drug. Moreover, CYP3A4 substantially contributes to the 5-sulfoxidation of chlorpromazine. These final conclusions are based on our consistent results of the Eadie–Hofstee analysis, correlation and inhibition studies, and on the demonstrated ability of cDNA-expressed CYP isoforms to metabolize chlorpromazine. Moreover, the present study conducted using of cDNA-expressed human CYP isoforms allows us for the first time to estimate the relative contribution of individual CYP enzymes to the 5-sulfoxidation, mono-N-demethylation and di-N-demethylation of chlorpromazine.

The results obtained with cDNA-expressed human CYP isoforms have shown that several enzymes generate detectable, but different, amounts of chlorpromazine 5-sulfoxide, while mono-N-desmethylchlorpromazine and di-N-desmethylchlorpromazine are formed by Supersome CYP1A2 at a therapeutic concentration of the drug only. Nevertheless, in the liver, the amount of the metabolite formed by an individual CYP isoform depends on both its catalytic activity with respect to product formation and its relative contribution to the total CYP content. Therefore we roughly estimated the contribution of the CYP enzymes studied to chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-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 CYP content in human liver. The calculations made at a low (therapeutic) concentration of chlorpromazine (10 μM) indicate that CYP1A2 is the only CYP isoform that catalyzes the mono-N-demethylation and di-N-demethylation of chlorpromazine (100%) and is the main isoform responsible for chlorpromazine 5-sulfoxidation (64%). CYP3A4 contributes to a lesser degree to chlorpromazine 5-sulfoxidation (34%). The role of CYP2B6, CYP2C19 and CYP2D6 in the catalysis of the latter reaction seems negligible (0.1–2%) (Table 3). Similar results were obtained at a higher, non-therapeutic concentration of the drug (100 μM); however, the contribution of CYP1A2 to chlorpromazine mono-N-demethylation was noticeably lower (75%) compared to 10 μM chlorpromazine, mostly in favour of CYP2C19 and CYP3A4 (about 12% each) (Table 3).

Recently there have been described several prediction methods for assessing the contribution of CYP isoenzymes to certain metabolic reactions in human liver microsomes [19–21]. At least two of them seem relevant: one based on the abundance of the CYP protein in human liver microsomes, used for our study, and the other founded on the relative activity factor (RAF = V_{max} of the specific reaction in liver microsomes/V_{max} of the specific reaction in cDNA-expressed CYP isoform). However, irrespective of the calculation methods (e.g., RAF or the abundance method), in the present study the contribution of CYP1A2 to chlorpromazine mono-N-demethylation and di-N-demethylation was always 100%, since the above reactions were catalyzed exclusively by CYP1A2 at a therapeutic concentration of the drug. In the case of chlorpromazine 5-sulfoxidation, our results (based on the abundance method), are in line with the inhibition study showing that furafylline and ketoconazole decrease the rate of chlorpromazine 5-sulfoxidation to the extent that confirms the calculated values of CYP1A2 and CYP3A4 contribution to this metabolic pathway; these results also agree with the findings of a correlation study.

The present results are in line with our earlier data [22–24] which showed the main contribution of CYP1A2 and CYP3A4 to the 5-sulfoxidation (in the thiazine ring) of the phenothiazine neuroleptics at therapeutic drug concentrations (the aliphatic-

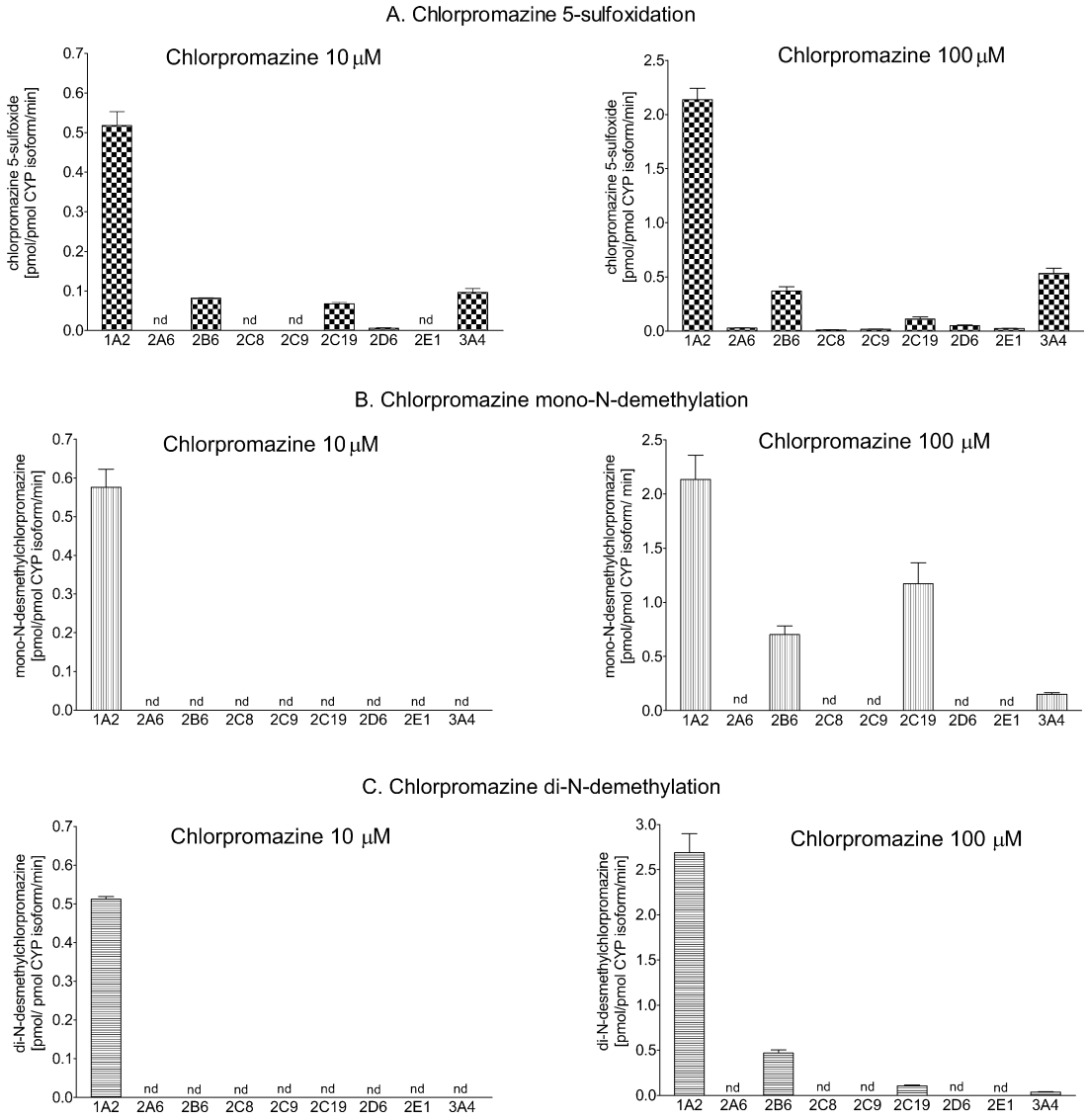


Fig. 5. The biotransformation of chlorpromazine via 5-sulfoxidation (A), mono-N-demethylation (B) and di-N-demethylation (C) by cDNA-expressed human CYP isoforms (Supersomes). Chlorpromazine (10 or 100 μM) was incubated with Supersomes (100 pmol of CYP/ml) and NADPH (1 mM) for 25 min. Each bar represents the mean value ± s.d. of three determinations from independent incubations, nd – not detected.

type phenothiazine neuroleptic promazine – 31% and 39%, respectively; the piperazine-type neuroleptic perazine – 32% and 30%, respectively; the piperidine-type neuroleptic thioridazine – 46% and 34%, respectively) (Table 4). As to promazine, perazine and thioridazine, the 5-sulfoxidation of chlorpromazine in the thiazine ring was also mediated by CYP1A2 (64%) and CYP3A4 (34%); however, the contribution of CYP1A2 to chlorpromazine 5-sulfoxidation was considerably higher compared to the previously tested phenothiazines. Significant qualitative and quantitative inter-drug differences in the contribution of CYP isoform to the metabolism of phenothiazines were observed in the catalysis of a side-chain mono-N-demethylation (Table 4). Promazine mono-N-demethylation was catalyzed to a similar extent by CYP1A2 and CYP2C19 (35% and 32%, respectively), while CYP2C19 was the main isoenzyme catalyzing perazine mono-N-demethylation (68%). On the other hand, CYP2C19 only marginally contributed to thioridazine mono-N-demethylation (4%). The latter metabolic pathway of thioridazine was mediated by CYP1A2 (44%) and CYP3A4 (52%). In contrast, in the present study, CYP1A2 was the only enzyme involved in chlorpromazine mono-N-demethylation. The above

Table 2
Kinetic parameters of chlorpromazine metabolism in cDNA-expressed human CYP isoforms (Supersomes).

CYPs	Chlorpromazine 5-sulfoxidation			Chlorpromazine mono-N-demethylation			Chlorpromazine di-N-demethylation		
	K_m[a]	V_{max}[b]	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m
CYP1A2	37	2.59	**0.070**	40	2.78	**0.069**	67	4.00	**0.060**
CYP2B6	61	0.58	0.009	115	0.76	0.007	88	0.80	0.009
CYP2C19	44	0.125	0.003	133	0.42	0.003	133	0.22	0.002
CYP3A4	57	1.24	**0.022**	100	0.27	0.003	89	0.07	0.001

[a] K_m expressed in μM.
[b] V_{max} expressed in pmol/mg protein/min.

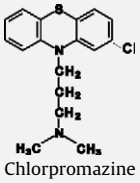
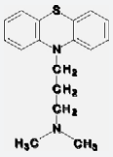
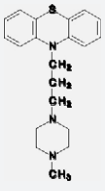
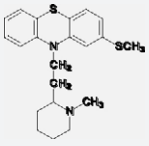
Table 3
Estimation of the contribution of CYP isoforms to the particular metabolic pathways of chlorpromazine on the basis of the rates of these reactions in Supersomes and average CYP isoform contents in the liver.

CYPs	Relative contribution of the isoform to the total CYP contents in liver microsomes [fraction]*	Relative contribution of the isoform to chlorpromazine metabolism in liver microsomes [percentage]**					
		Chlorpromazine 5-sulfoxidation		Chlorpromazine mono-N-demethylation		Chlorpromazine di-N-demethylation	
		Chlorpromazine		Chlorpromazine		Chlorpromazine	
		10 μM	100 μM	10 μM	100 μM	10 μM	100 μM
CYP1A2	0.127[(a)]	63.9	62.1	100	75.1	100	95.7
CYP2A6	0.040[(a)]	nd	0.2	nd	nd	nd	nd
CYP2B6	0.002[(a)]	0.2	0.2	nd	0.4	nd	0.2
CYP2C8	0.065[(b)]	nd	0.2	nd	nd	nd	nd
CYP2C9	0.197[(b)]	nd	0.7	nd	nd	nd	nd
CYP2C19	0.038[(b)]	1.9	1.0	nd	12.3	nd	1.1
CYP2D6	0.015[(a)]	0.1	0.2	nd	nd	nd	nd
CYP2E1	0.066[(a)]	nd	0.3	nd	nd	nd	nd
CYP3A4	0.288[(a)]	33.9	35.1	nd	12.2	nd	3.0

nd, not detected.
* Data according to Shimada et al. [29][(a)], Rostami-Hodjegan and Tucker [30][(b)].
** Relative contribution of CYPs to the particular metabolic pathways of chlorpromazine 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. 3) by the relative contribution of isoform to the total CYP content in liver microsomes. For details see Wójcikowski et al. [22].

differences may stem from individual chemical structures of the side chains and the presence of specific substituents in the aromatic ring of the phenothiazines studied (Table 4), which influence their access to and the interaction with the catalytic sites of CYP isoforms. The lack of contribution of CYP2C19 or CYP3A4 to chlorpromazine mono-N-demethylation in a side chain (compared with promazine, perazine or thioridazine) may be due to some structural requirements of the enzyme for its substrates and to

Table 4
Phenothiazine structures and the main CYP isoforms contributing to their 5-sulfoxidation and mono-N-demethylation.

Phenothiazine	Side chain	Substituent in the aromatic ring	Contribution of CYP isoforms	
			5-sulfoxidation	Mono-N-demethylation
 Chlorpromazine	Aliphatic-type	–Cl	CYP1A2 (64%) CYP3A4 (34%)	CYP1A2 (100%)
 Promazine[a]	Aliphatic-type	–	CYP1A2 (31%) CYP3A4 (39%)	CYP1A2 (35%) CYP2C19 (32%)
 Perazine[b]	Piperazine-type	–	CYP1A2 (32%) CYP3A4 (30%)	CYP2C19 (68%)
 Thioridazine[c]	Piperidine-type	–S–CH_3	CYP1A2 (46%) CYP3A4 (34%)	CYP1A2 (44%) CYP3A4 (52%)

Data according to:
[a] Wójcikowski et al. [22].
[b] Wójcikowski et al. [23].
[c] Wójcikowski et al. [24].

limited space around the nitrogen in a side chain by the bulkiness of the chlorine atom which hinders oxidation of the chemically vulnerable position by CYP2C19 and CYP3A4. Thus promazine which differs from chlorpromazine in the absence of the chlorine substituent in the aromatic ring only, allows CYP2C19 to catalyze mono-N-demethylation.

Our data on the main contribution of CYP1A2 to the metabolism of chlorpromazine may have significant implications for the prediction of drug-drug interactions. Chlorpromazine is administered for months or years, very often to patients treated simultaneously with other clinically important drugs that are substrates for CYP1A2 (e.g., caffeine, theophylline, phenacetin, amitriptyline, imipramine, propranolol clozapine, and melatonin). Considering the serious side effects of chlorpromazine and some of its metabolites, a metabolic interaction between this neuroleptic and other drugs may be of clinical importance. Metabolic interactions of this type between chlorpromazine and antidepressant drugs have been found in rats [16] and their serious consequences have been reported in humans [25]. Loga et al. [25] showed a significant elevation of plasma chlorpromazine concentration after concomitant amitriptyline administration to schizophrenic patients. That increase was accompanied with further amitriptyline-induced potentiation of the diminished blood pressure, observed in patients treated with chlorpromazine. Moreover, since phenothiazine neuroleptics are combined with antidepressants for the treatment of complex psychiatric disorders (e.g., delusional depression), and selective serotonin reuptake inhibitors (e.g., fluvoxamine) are used as antidepressants that attenuate negative symptoms of schizophrenia [26,27], the CYP1A2-mediated chlorpromazine metabolism may be inhibited by fluvoxamine, a potent inhibitor of that enzyme [28].

In summary, the results of the present study show that (1) CYP1A2 is the only CYP isoform that catalyzes the mono-N-demethylation and di-N-demethylation of chlorpromazine, and is the main isoform responsible for chlorpromazine 5-sulfoxidation at a therapeutic concentration of the drug; (2) besides CYP1A2, CYP3A4 substantially contributes to chlorpromazine 5-sulfoxidation; (3) similar results have been obtained at a higher, non-therapeutic concentration of the drug (100 μM); however, the contribution of CYP1A2 to chlorpromazine mono-N-demethylation is noticeably smaller compared to 10 μM chlorpromazine, mostly in favour of CYP2C19 and CYP3A4.

In conclusion, the catalysis of chlorpromazine N-demethylation and 5-sulfoxidation in humans exhibits a stricter CYP1A2 preference compared to the previously tested phenothiazines. Different structures of the phenothiazine neuroleptics tested (the structure of a side chain and the presence of a specific substituent in the aromatic ring) influence their interactions with the catalytic sites of cytochrome P450. Hence the knowledge of the enzyme catalysis of the metabolism of a particular phenothiazine neuroleptic (e.g., chlorpromazine) may help to avoid undesirable drug interactions.

Acknowledgements

The study was supported by grant no. N N405 182435 from the Ministry of Science and Higher Education, Warszawa, Poland, and by statutory funds from the Institute of Pharmacology, the Polish Academy of Sciences in Kraków. Thanks are due to Ms. Justyna Staryńska for her excellent technical assistance.

References

[1] Liu X, De Haan S. Chlorpromazine dose for people with schizophrenia. Cochrane Database Syst Rev 2009;2:CD007778.
[2] Bylund DB. Interactions of neuroleptic metabolites with dopaminergic, alpha adrenergic and muscarinic cholinergic receptors. J Pharmacol Exp Ther 1981;217:81–6.
[3] Hals PA, Hall H, Dahl SG. Phenothiazine drugs metabolites: dopamine D_2 receptor, α_1 and α_2-adrenoceptor binding. Eur J Pharmacol 1986;125:373–81.
[4] Palmer GC, Blosser JC, McCreddy SA, Barrantes A, Manian AA. Correlation of activity of chlorpromazine and respective hydroxy, dimethoxy and sulphoxide analogues on dopamine, muscarinic, histamine and calmoduline sites of action. Xenobiotica 1988;18:277–89.
[5] Hartmann F, Gruenke LD, Craig JC, Bissel M. Chlorpromazine metabolism in extracts of liver and small intestine from guinea pig and from man. Drug Metab Dispos 1983;3:244–8.
[6] Jørgensen A. Metabolism and pharmacokinetics of antypsychotic drugs. In: Bridges JW, Chasseaud LF, editors. Progress in drug metabolism. Taylor & Francis Ltd.; 1986. p. 111–74.
[7] Midha KK, Hubbard JW, Cooper JK, Gurnsey T, Hawes EM, McKay G, et al. Therapeutic monitoring of chlorpromazine. IV: comparison of new high-performance liquid chromatographic method with radioimmunoassays for parent drug and some of its major metabolites. Ther Drug Monit 1987;3:358–65.
[8] Chetty M, Moodley SV, Miller R. Important metabolites to measure in pharmacodynamic studies of chlorpromazine. Ther Drug Monit 1994;1:30–6.
[9] Daniel W. Metabolism of psychotropic drugs: pharmacological and clinical relevance. Pol J Pharmacol 1995;47:367–79.
[10] Chetty M, Pillay VL, Moodley SV, Miller R. Response in chronic schizophrenia correlated with chlorpromazine, 7-OH-chlorpromazine and chlorpromazine sulfoxide levels. Eur Neuropsychopharmacol 1996;2:85–91.
[11] Chetty M, Gouws E, Miller R, Moodley SV. The use of a side effects as a qualitative indicator of plasma chlorpromazine levels. Eur Neuropsychopharmacol 1999;1–2:77–82.
[12] Yoshii K, Kobayashi K, Tsumuji M, Tani MS, Shimada N, Chiba K. Identification of human cytochrome P450 isoform involved in the 7-hydroxylation of chlorpromazine by human liver microsomes. Life Sci 2000;67:175–84.
[13] Cashman JR, Yang Z, Yang L, Wrighton S. Stereo- and regioselective N- and S-oxidation of tertiary amines and sulfides in the presence of adult human liver microsomes. Drug Metab Dispos 1993;21:492–501.
[14] Tay JK, Tan CH, Chong SA, Tan EC. Functional polymorphism of the cytochrome P450 1A2 (CYP1A2) gene and prolonged QTc interval in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2007;6:1297–302.
[15] Chetty M, Miller R, Moodley SV. Smoking and body weight influence the clearance of chlorpromazine. Eur J Clin Pharmacol 1994;6:523–6.
[16] Daniel W, Melzacka M. The effect of neuroleptics on imipramine demethylation in rat liver microsomes and imipramine and desipramine level in the rat brain. Biochem Pharmacol 1986;19:3249–53.
[17] Lemoine A, Gautier JC, Azoulay D, Kiffel L, Belloc C, Guengerich FP, et al. Major pathway of imipramine metabolism is characterized by cytochrome P-450 1A2 and P-450 3A4 in human liver. Mol Pharmacol 1993;43:827–32.
[18] Yeung PK, Hubbard JW, Cooper JK, Midha KK. A study of the kinetics of chlorpromazine sulfoxide by a specific radioimmunoassay after a single oral dose of chlorpromazine in healthy volunteers. J Pharmacol Exp Ther 1983;3:833–8.
[19] Rodrigues AD. Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. Biochem Prarmacol 1999;57:465–80.
[20] Nakajima M, Nakamura S, Tokudome S, Shimada N, Yamazaki H, Yokoi T. Azelastine N-demethylation by cytochrome P-450 (CYP)3A4, CYP2D6, and CYP1A2 in human liver microsomes: evaluation of approach to predict the contribution of multiple CYPs. Drug Metab Dispos 1999;27:1381–91.
[21] Venkatakrishnan K, von Moltke L, Court MH, Harmatz JS, Crespi CL, Greenblatt DJ. Comparison between cytochrome P450 (CYP) content and relative activity approaches to scaling from cDNA-expressed CYPs to human liver microsomes: ratios of accessory proteins as sources of discrepancies between the approaches. Drug Metab Dispos 2000;28:1493–504.
[22] Wójcikowski J, Pichard-Garcia L, Maurel P, Daniel WA. Contribution of human cytochrome P-450 isoforms to the metabolism of the simplest phenothiazine neuroleptic promazine. Br J Pharmacol 2003;138:1465–74.
[23] Wójcikowski J, Pichard-Garcia L, Maurel P, Daniel WA. The metabolism of the piperazine-type phenothiazine neuroleptic perazine by the human cytochrome P-450 isoenzymes. Eur Neuropsychopharmacol 2004;14:199–208.
[24] Wójcikowski J, Maurel P, Daniel WA. Characterization of human cytochrome P450 enzymes involved in the metabolism of the piperidine-type phenothiazine neuroleptic thioridazine. Drug Metab Dispos 2006;3:471–6.
[25] Loga S, Curry S, Lader M. Interaction of chlorpromazine and nortryptyline in patients with schizophrenia. Clin Pharmacokinet 1981;6:454–62.
[26] Nelson JC. Combined treatment strategies in psychiatry. J Clin Psychiat 1993;54(Suppl. 9):42–9.
[27] Silver H, Nassar A. Fluvoxamine improves negative symptoms in treated chronic schizophrenia: an add-on double-blind, placebo-controlled study. Biol Psychiat 1992;31:698–704.
[28] Brøsen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. Biochem Pharmacol 1993;45:1211–4.
[29] Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogenes and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 1994;270:414–23.
[30] Rostami-Hodjegan A, Tucker GT. Simulation and prediction of in vivo drug metabolism in human populations from in vitro data. Nat Rev Drug Discov 2007;6:140–8.