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Perazine at therapeutic drug concentrations inhibits human cytochrome P450 isoenzyme 1A2 (CYP1A2) and caffeine metabolism – an *in vitro* study

Jacek Wójcikowski, Władysława A. Daniel

Department of Pharmacokinetics and Drug Metabolism, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Correspondence: Jacek Wójcikowski, e-mail: wojcikow@if-pan.krakow.pl

Abstract:
The aim of the present study was to estimate the inhibitory effect of perazine, a phenothiazine neuroleptic with piperazine structure in a side chain, on human CYP1A2 activity measured as a rate of caffeine 3-N- and 1-N-demethylation. Moreover, the influence of perazine on other caffeine metabolic pathways such as 7-N-demethylation (CYP1A2, CYP2C8/9, CYP3A4) and 8-hydroxylation (CYP3A4, CYP1A2, CYP2C8/9) was also determined. The Dixon analysis showed that in both human liver microsomes and Supersomes CYP1A2 perazine potently and to a similar degree inhibited caffeine 3-N-demethylation (K_i = 3.5 μM) and 1-N-demethylation (K_i = 5 μM). Perazine moderately diminished the rate of caffeine 7-N-demethylation in Supersomes CYP1A2 (K_i = 11.5 μM) and liver microsomes (K_i = 20 μM), and attenuated C-8-hydroxylation (K_i = 15.5 μM) in Supersomes CYP1A2. On the other hand, perazine weakly inhibited caffeine C-8-hydroxylation in liver microsomes (K_i = 98 μM). About 80% of basal CYP1A2 activity was reduced by the therapeutic concentrations of perazine (5–10 μM).
The obtained results show that perazine at its therapeutic concentrations is a potent inhibitor of human CYP1A2. Hence, taking account of CYP1A2 contribution to the metabolism of endogenous substances (steroids), drugs (xanthine derivatives, phenacetin, propranolol, imipramine, phenothiazine neuroleptics, clozapine) and carcinogenic compounds, the inhibition of CYP1A2 by perazine may be of physiological, pharmacological and toxicological importance.

Key words:
perazine, caffeine metabolism, human CYP1A2, inhibition

Introduction

Cytochrome P450 isoenzyme 1A2 (CYP1A2) constitutes approximately 13% of the total cytochrome P450 (CYP) protein in human liver [35]. The enzyme catalyzes the metabolism of several clinically important drugs and endogenous substances, such as caffeine, theophylline, phenacetin, imipramine, phenothiazine neuroleptics, propranolol, clozapine, melatonin and steroids [30, 31, 40, 41]. It also plays an important role in the metabolic transformation of heterocyclic aromatic amines into reactive intermediates, leading to toxicity and cancer [28]. Furafylline, an antiasthmatic drug of the methylxanthine group, has been reported to be a selective and potent inhibitor of

CYP1A2 activity in human liver microsomes [6, 25, 34]. Moreover, it has been shown that fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), strongly inhibits human CYP1A2 [4]. Phenacetin *O*-deethylase, acetanilide 4-hydroxylase and caffeine 3-N-demethylase activities are often used as markers of human CYP1A2 activity [16, 27, 31, 38].

Caffeine (1,3,7-trimethylxanthine) is an established marker substrate for testing CYP1A2 activity using 3-N-demethylation in humans [3, 16, 24, 32, 34]. The compound undergoes 1-N-demethylation to theobromine, 3-N-demethylation to paraxanthine (the main metabolic route), 7-N-demethylation to theophylline and C-8-hydroxylation to 1,3,7-trimethyluric acid. Recent studies by Kot and Daniel [24] showed that, besides 3-N-demethylation, 1-N-demethylation is also specifically catalyzed by CYP1A2 at a therapeutic concentration of caffeine (100 μM), both reactions showing a simple enzyme kinetics. The 7-N-demethylation of caffeine is catalyzed non-specifically, mainly by CYP1A2, and to a lesser extent by CYP2C8/9 and CYP3A4. C-8-hydroxylation preferentially involves CYP1A2 and CYP3A4 and, to a lesser degree, CYP2C8/9. Similar results were obtained at a higher substrate concentration (1 mM). Caffeine may also be used as a marker substrate for assessing CYP1A2 activity in rats, but only in case when C-8-hydroxylation is used as a marker reaction [23].

Perazine belongs to the group of phenothiazine neuroleptics with piperazine structure in a side chain. It is a moderate antagonist of dopaminergic D_2 receptors and a weak antagonist of dopaminergic D_1, adrenergic α_1, serotonergic 5-HT_2 and cholinergic muscarinic M_1 receptors, hence it rarely produces side-effects in the central or the autonomic nervous system. In contrast to many other phenothiazine neuroleptics, perazine does not negatively influence mood; furthermore, some clinicians even attribute certain antidepressant properties to it. For these reasons, perazine is often used in geriatric patients and in a combination therapy with antidepressants [22, 29]. CYP1A2 and CYP3A4 are the main izoenzymes that catalyze 5-sulfoxidation, while CYP2C19 is the chief isoform responsible for the N-demethylation of perazine in humans [43]. Unlike in humans, CYP2B and CYP2D are the main isoforms responsible for perazine 5-sulfoxidation, while CYP1A2, CYP2B and CYP3A chiefly catalyze its demethylation in rats [11]. On the other hand, the aromatic hydroxylation of phenothiazines is governed by CYP2D6 in humans [26, 44].

Our preliminary study, performed on a primary culture of human hepatocytes derived from one patient, suggested an inhibitory effect of perazine on CYP1A2 activity [42]. Therefore the aim of the present study was to estimate the inhibitory effect (K_i) of perazine on human CYP1A2 activity (measured as a rate of caffeine 3-N-demethylation and 1-N-demethylation) using two complementary *in vitro* models: human liver microsomes (pooled liver microsomes from six patients) and cDNA-expressed human CYP1A2 (Supersomes CYP1A2). Moreover, the influence of perazine on other caffeine metabolic pathways (7-N-demethylation and 8-hydroxylation) was concurrently determined.

Materials and Methods

Drugs and chemicals

Perazine (dimaleate) was obtained from Labor (Wrocław, Poland). Caffeine, paraxanthine, theobromine, theophylline, 1,3,7-trimethyluric acid and NADPH were provided by Sigma (St. Louis, USA). All the HPLC purity organic solvents were supplied by Merck (Darmstadt, Germany).

Human liver microsomes

Pooled human liver microsomes from patients HG5, HG8, HG15, HG6, HG83 and HG85 were obtained from Gentest Co. (Woburn, MA, USA).

Studies into caffeine metabolism in human liver microsomes were carried out at the linear dependence of product formation on time, protein and substrate concentration. The rates of 1-N-, 3-N- and 7-N-demethylation and 8-hydroxylation of caffeine (caffeine concentrations: 50, 100, 200, 400 and 800 μM) were assessed in the absence and presence of perazine added *in vitro* (perazine concentrations: 1, 2.5, 5, 10, 20, 50 μM). Incubation was carried out in a system containing liver microsomes (ca. 0.5 mg of protein/ml), a phosphate buffer (0.15 M, pH 7.4) and NADPH (1 mM). The final incubation volume was 0.5 ml. Each sample was prepared in duplicate. After 50-min incubation, the reaction was terminated by adding 700 μl of a 2% $ZnSO_4$ and 50 μl of 2M HCl. Caffeine and its metabolites were analyzed by the

high-performance liquid chromatography method (HPLC) described below.

cDNA-expressed human CYPs

Microsomes from baculovirus-infected insect cells expressing CYP1A2 co-expressed with NADPH P450 oxidoreductase (Supersomes 1A2) were obtained from Gentest Co. (Woburn, MA, USA). Studies into caffeine metabolism in Supersomes were carried out at the linear dependence of product formation on time, protein and the amount of CYP1A2 and substrate concentration. Caffeine metabolism was studied under experimental conditions similar to those described for liver microsomes using 50, 100, 200 and 400 μM caffeine and 0.5, 1, 2.5, 5, 10 and 20 μM perazine, except for the fact that the final concentration of CYP1A2 was 100 pmol/ml. Caffeine and its metabolites were analyzed by the HPLC method described below.

Determination of caffeine and its metabolites

Caffeine and its four primary metabolites: theobromine (caffeine 1-N-demethylation), paraxanthine (caffeine 3-N-demethylation), theophylline (caffeine 7-N-demethylation) and 1,3,7-trimethyluric acid (caffeine C-8-hydroxylation), were assessed using the HPLC method as described previously [24]. Briefly, after incubation, the samples were centrifuged for 10 min at 2000 × g. A water phase containing caffeine and its metabolites was extracted with 6 ml of an organic mixture consisting of ethyl acetate and 2-propanol (8:1, v/v). The residue obtained after evapo-

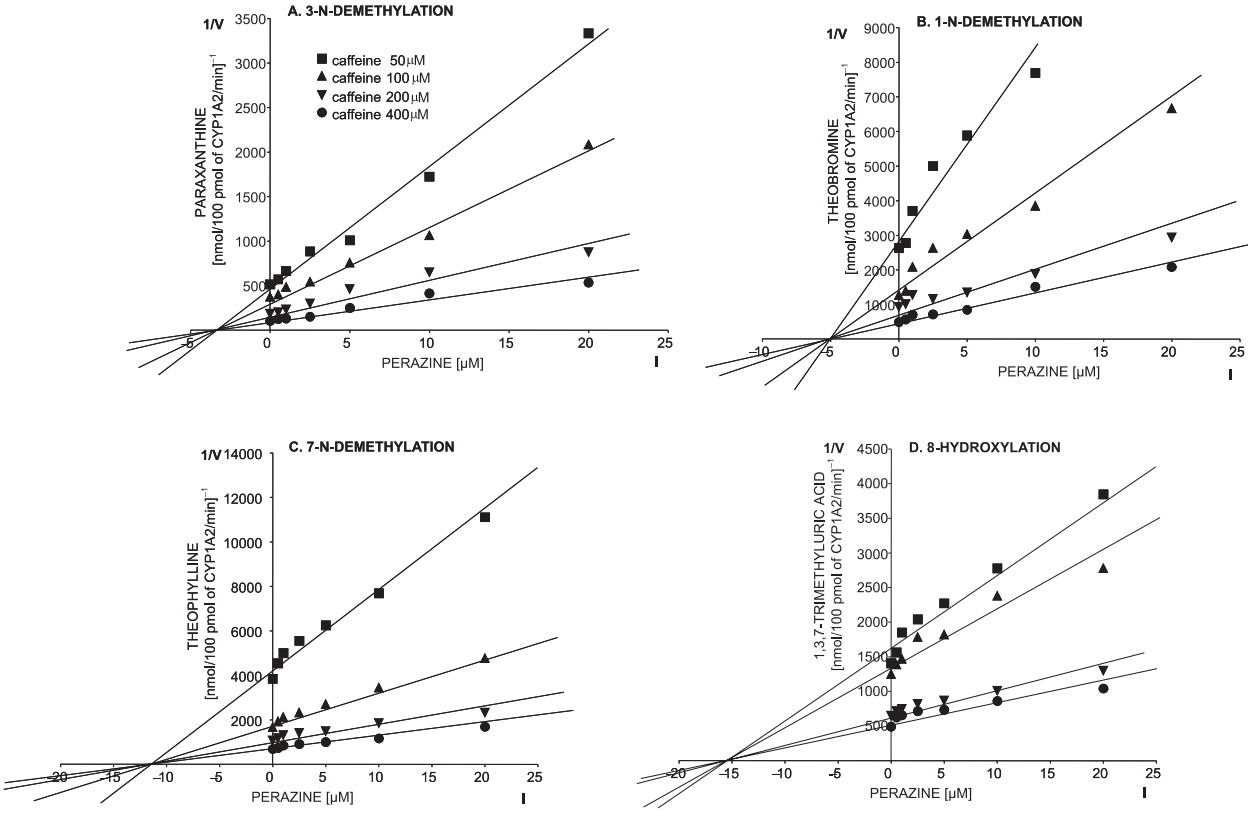

Fig. 1. The influence of perazine on the metabolism of caffeine in human cDNA expressed CYP1A2 (Supersomes CYP1A2): (**A**) caffeine 3-N-demethylation; (**B**) caffeine 1-N-demethylation; (**C**) caffeine 7-N-demethylation; (**D**) caffeine C-8-hydroxylation (Dixon's plots). Each point represents the mean value of two independent analyses. K_i values are shown in Table 1. V velocity of the reaction, I concentration of the inhibitor (perazine)

ration 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 La Chrom Merck-Hitachi (Darmstadt, Germany) HPLC system, equipped with a L-7100 pump, an L-7400 UV detector and a D-7000 System Manager, was used. The analytical column (Supelcosil LC-18, 150 × 4.6 mm, 5 µm) was from Supelco (Bellefonte, USA). The mobile phase consisted of 0.01 M acetate buffer (pH = 3.5) and methanol (91:9, v/v). The flow rate was 1 ml/min (0–16.5 min) followed by 3 ml/min (16.6–25 min). The column temperature was 30°C. The absorbance of caffeine and its metabolites was measured at a wavelength of 270 nm.

Results

Perazine significantly inhibited caffeine 1-N-, 3-N-, 7-N-demethylation and C-8-hydroxylation in human liver microsomes and Supersomes CYP1A2; however, its potency towards particular metabolic pathways was diverse. The Dixon analysis showed that in both human liver microsomes and Supersomes CYP1A2, perazine potently and to a similar degree inhibited caffeine 3-N-demethylation (K_i = 3.5 µM) and 1-N-demethylation (K_i = 5 µM) (Figs. 1 and 2, Tab. 1). Perazine moderately diminished the rate of caffeine 7-N-demethylation in Supersomes CYP1A2

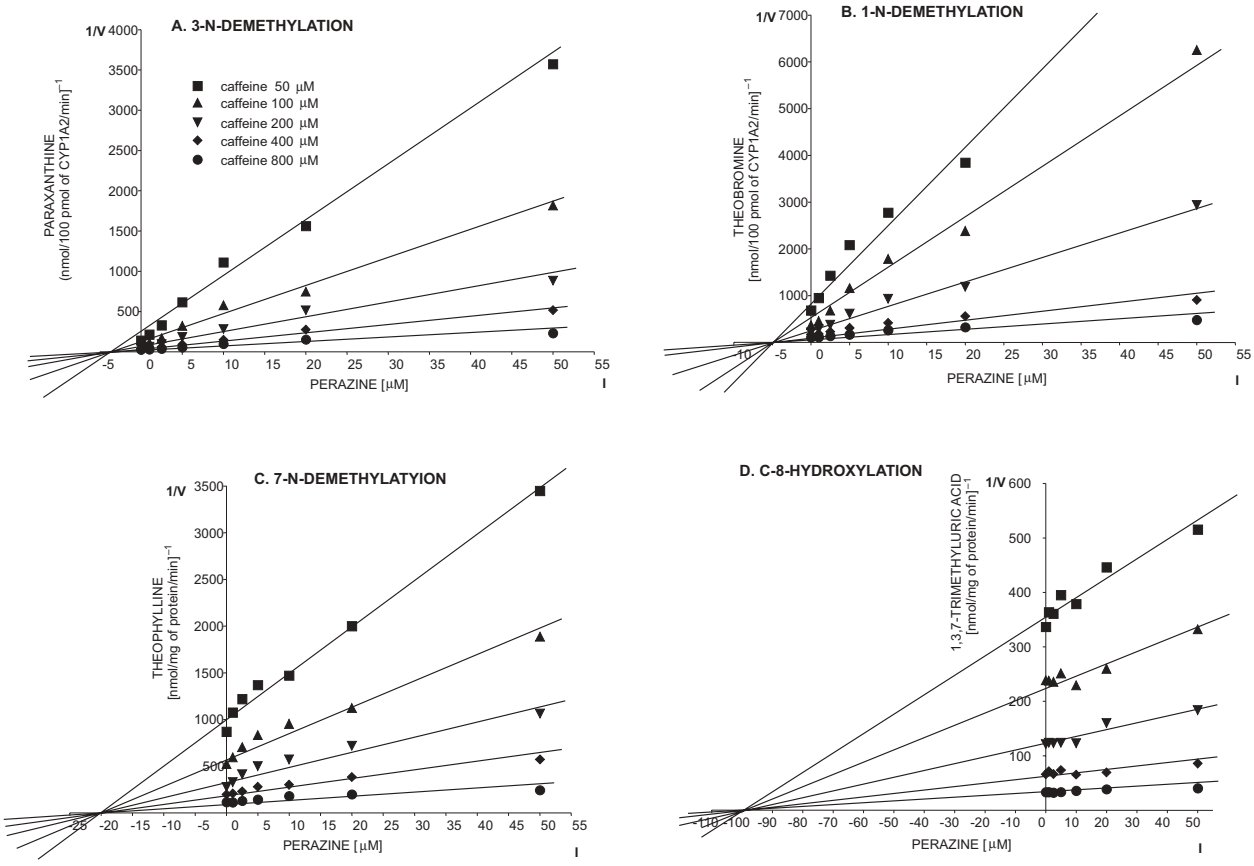

Fig. 2. The influence of perazine on the metabolism of caffeine in human liver microsomes: (**A**) caffeine 3-N-demethylation; (**B**) caffeine 1-N-demethylation; (**C**) caffeine 7-N-demethylation; (**D**) caffeine C-8-hydroxylation (Dixon's plots). Each point represents the mean value of two independent analyses. K_i values are shown in Table 1. V velocity of the reaction, I concentration of the inhibitor (perazine)

Tab. 1. The influence of perazine on the metabolism of caffeine *in vitro*

Enzyme preparations	Inhibition of caffeine metabolism by perazine K_i (μM)			
	Caffeine 3-N-demethylation (Paraxanthine)	Caffeine 1-N-demethylation (Theobromine)	Caffeine 7-N-demethylation (Theophylline)	Caffeine C-8-hydroxylation (1,3,7-trimethyluric acid)
Human liver microsomes	3.5	5	20	98
cDNA-expressed CYP1A2 (Supersomes)	3.5	5	11.5	15.5

The presented inhibition constants (K_i) for inhibition of particular metabolic pathway were graphically calculated using Dixon analysis

(K_i = 11.5 μM) and in liver microsomes (K_i = 20 μM), as well as the rate of caffeine C-8-hydroxylation (K_i = 15.5 μM) in Supersomes CYP1A2 (Figs. 1 and 2, Tab. 1). On the other hand, perazine weakly inhibited caffeine C-8-hydroxylation in liver microsomes (K_i = 98 μM) (Fig. 2, Tab. 1).

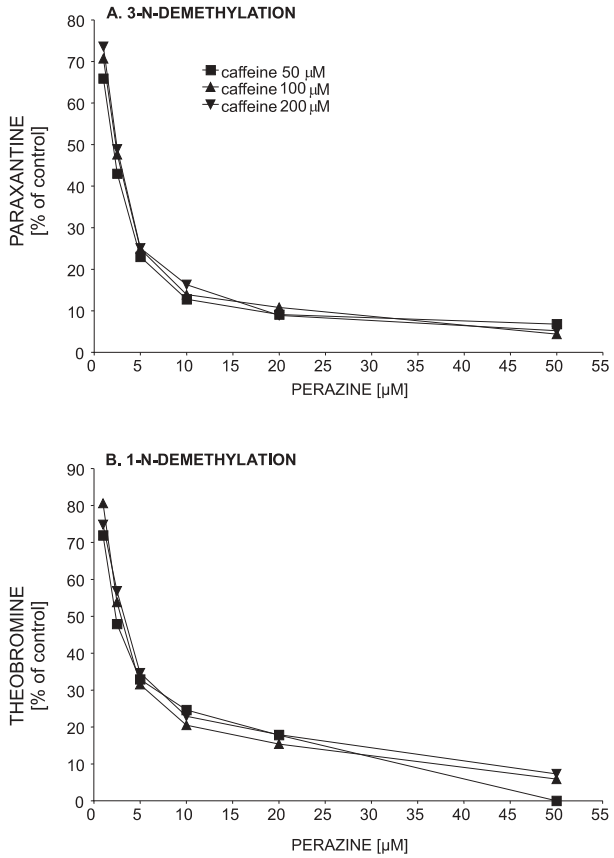

Fig. 3. A concentration-dependent effect of perazine on CYP1A2 activity measured as a rate of the 3-N-demethylation (**A**) and 1-N-demethylation (**B**) of caffeine in human liver microsomes. Each point represents the mean value of two independent analyses

Perazine inhibited in a concentration-dependent manner CYP1A2 activity measured as a rate of caffeine 3-N- and 1-N-demethylation in liver microsomes (Fig. 3). About 80% of basal CYP1A2 activity was distinctly inhibited at the therapeutic concentrations of perazine (5–10 μM) and caffeine (up to 100 μM). Higher concentrations of perazine did not significantly enhance that effect (Fig. 3).

Discussion

The obtained results show that perazine applied in therapeutic concentrations exerts an inhibitory effect on caffeine metabolism in human, its potency towards particular metabolic pathways being different, though. The Dixon analysis of caffeine metabolism, carried out on human liver microsomes and cDNA-expressed CYP1A2 (Supersomes CYP1A2), showed that perazine decreased the rate of 1-N-, 3-N- and 7-N-demethylation, and C-8-hydroxylation of caffeine, the effect on 3-N-demethylation and 1-N-demethylation being the most pronounced (K_i = 3.5–5 μM). This observation indicates potent inhibition of CYP1A2 by perazine at the K_i value in the range observed for the K_i values of such well-known CYP1A2 inhibitors as furafylline and fluvoxamine (K_i = 0.12–3 μM, depending on the substrate used), as well as in the range for the therapeutic concentrations of the neuroleptic tested [1, 4, 6, 15, 25, 34, 39].

A number of studies into caffeine metabolism have shown that 3-N-demethylation to paraxanthine in humans (the main oxidation pathway) is specifically catalyzed by CYP1A2. However, recent detailed studies by Kot and Daniel [24] have demonstrated that CYP1A2 is the chief enzyme that catalyzes not only

3-N-demethylation, but also 1-N-demethylation of caffeine (an 85 and 75% contribution, respectively) at the therapeutic concentration of caffeine (100 μM) in humans. It also substantially contributes to 7-N-demethylation (39%) and C-8-hydroxylation (29%). Moreover, 7-N-demethylation is catalyzed by CYP2C8/9 (25%) and CYP3A4 (14%), while C-8-hydroxylation is substantially mediated by CYP3A4 (30%), and to a lesser extent by CYP2C8/9 (18%). At a higher concentration of the substrate (1 mM), only CYP1A2 contribution to the C-8-hydroxylation of caffeine is visibly decreased (to 16%), mostly in favor of CYP2C8/9 (29%). The present study shows that perazine potently inhibits 1-N-demethylation (K_i = 5 μM in liver microsomes and Supersomes CYP1A2) and moderately lowers 7-N-demethylation (K_i = 20 μM in liver microsomes, and 11.5 μM in Supersomes CYP1A2). Moreover, perazine moderately diminishes caffeine C-8-hydroxylation in liver microsomes (K_i = 15.5 μM), but weakly inhibits this pathway in Supersomes CYP1A2 (K_i = 98 μM). In the light of Kot and Daniel's findings [24], the obtained differences in the K_i values for caffeine 7-N-demethylation and C-8-hydroxylation between liver microsomes (containing all liver CYP isoforms) and Supersomes (containing CYP1A2 only) may stem from the non-specific catalysis of these metabolic pathways (by CYP1A2, CYP2C8/9 and CYP3A4) in liver microsomes. In contrast, caffeine 3-N-demethylation and 1-N-demethylation are specifically catalyzed by CYP1A2 in liver microsomes. Hence perazine inhibits the latter metabolic pathways with a similar potency in liver microsomes and Supersomes CYP1A2.

Although the therapeutic plasma concentration of perazine reaches up to 0.5 μM, its concentration in the liver may be about 10–15 times higher (up to 10 μM) than in the plasma owing to drug distribution [1, 15, 39]. Hence, the interaction between perazine and CYP1A2 *in vitro*, observed in the present study, should also be observed *in vivo*, since the calculated K_i values are below the presumed concentration range for perazine in the liver *in vivo*, in both pharmacological experiments and psychiatric patients. Accordingly, a recent study by Schaller et al. [33] showed a significant elevation of serum clozapine level (up to 400% of the control value) after concomitant perazine administration to schizophrenic patient. The latter authors suggested that the observed effect was produced by inhibition of hepatic CYP1A2 (the main isoenzyme responsible for clozapine metabolism) and/or CYP3A4 by perazine. However, our earlier studies demonstrated week inhibition of human CYP3A4 by perazine [42]. Thus the observed interaction between clozapine and perazine seems to stem exclusively from CYP1A2 inhibition by perazine. Perazine may also be responsible for metabolic interactions with antidepressants (tricyclic, SSRIs). Interactions of this type between perazine and tricyclic antidepressants or SSRIs have been observed in rats [7, 12, 13]. However, it is not unlikely that metabolic interactions between perazine and other psychotropic drugs may also involve the CYP2D subfamily. As shown previously, perazine exerts relatively potent inhibition of rat CYP2D (K_i = 18 μM), which may suggest potent inhibition of human CYP2D6 by that neuroleptic [8, 9]. The above observation is consistent with the results obtained by Shin et al. [36], which show strong inhibition of CYP2D6 (K_i = 0.8 μM) by perphenazine, another piperazine-type neuroleptic.

Despite its potent inhibition of human CYP1A2, perazine exerts a weaker inhibitory effect on rat CYP1A2 (K_i = 52 μM), which indicates species-related differences in CYP1A2 structure and function [10, 14]. These results are in line with those obtained by Sesardic et al. [34], who showed that furafylline inhibited human CYP1A2 1000-times more potently than rat CYP1A2 one. Therefore, the rat does not seem to be a suitable animal model for studying drug interactions with CYP1A2.

Caffeine, a component of coffee, tea, energy drinks and numerous drugs, is a purine alkaloid and the most universally used psychoactive substance. The caffeine concentration value of approximately 100 μM may be considered "a maximal therapeutic concentration in humans". However, some individuals can consume more than 1 g/day (about 15 mg/kg/day) and even up to 3.5 g/day (about 50 mg/kg/day) of caffeine in a caffeinism syndrome which leads to caffeine concentrations above 100 μM in their blood plasma [2, 5, 21]. Since CYP1A2 is the main isoform responsible for caffeine metabolism, a pharmacokinetic interaction between caffeine and perazine may occur. Caffeine acts through multiple mechanisms, the most important of them being antagonism of adenosine receptors (A_1 and A_{2A}), observed at its therapeutic concentrations (10–100 μM). As an adenosine receptor antagonist, caffeine increases the release of various neurotransmitters [18]. Moreover, as a result of a negative interaction between adenosine and dopaminergic receptors, caffeine enhances responses from dopaminer-

gic receptors [19]. Due to its ability to affect neurotransmission in different regions of the brain, caffeine displays psychomotor stimulant properties and promotes behavioral functions such as vigilance, attention, mood, and arousal [17]. Since caffeine is not only a metabolic marker substance, but also a drug with a very broad pharmacological spectrum, both its metabolic and pharmacodynamic properties must be considered when caffeine-perazine interactions are predicted in pharmacological experiments with laboratory animals and under clinical conditions. For example, fluvoxamine, a substrate and a strong CYP1A2 inhibitor, decreases the activity of CYP1A2, which may lead to caffeine intoxication [20]. Similarly, it has been reported that caffeine accumulates up to a toxic level due to the potent inhibition of its metabolism in coffee drinking furafylline-treated volunteers [37].

In conclusion, the obtained results show that perazine at therapeutic concentrations is a potent inhibitor of human CYP1A2. The results of the present study may be of great practical value, since perazine is administered to patients for months or even years, very often in combination with antidepressants (tricyclic, SSRIs) or other neuroleptics. Thus, considering the contribution of CYP1A2 to the metabolism of endogenous substances (e.g. steroids), drugs and carcinogenic compounds, CYP1A2 inhibition by perazine may be of physiological, pharmacological and toxicological importance.

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