Innov Biosyst Bioeng, 2025, vol. 9, no. 2, 4–11
doi: 10.20535/ibb.2025.9.2.309378

Original article

INHIBITION OF CYTOCHROME P450 ACTIVITIES BY PROPOXAZEPAM: SAFETY ASSESSMENT IN CONTEXT FOR POTENTIAL DRUG INTERACTIONS

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Received 31 July 2024; Accepted 6 December 2024

Background. Propoxazepam is a new anelgetic agent of the benzodiazepine group, chemically known as 7-bromo-5-(o-chlorophenyl)-3-propyloxy-1,2-dihydro-3H-1,4-benzodiazepine-2-one. Propoxazepam is considered a possible substrate of the CYP system, so its effect of on the CYP3A4 enzyme activity was investigated *in vitro* using human liver microsomes.

Objective. To evaluate the effects of propoxazepam on CYP3A4 activity *in vitro* using testosterone and midazolam as markers of metabolic activity for CYP3A4 in human liver microsomes.

Methods. Midazolam (1′-hydroxylation reaction) and testosterone (6β-hydroxylation reaction) were used as markers for CYP3A4-mediated activity. Ketoconazole (0.2 μM) was used as a positive control for reversible inhibition, and troleandomycin (50 μM) for metabolism-dependent inhibition. For reversible inhibition, propoxazepam was added together with the corresponding substrate and cofactor (NADPH), while for metabolism-dependent inhibition, it was incubated with microsomes and cofactor for 30 minutes prior to substrate addition.

Results. Propoxazepam at various concentrations (0 to 100 μM) consistently inhibited CYP3A4 activities for both substrates, showing a similar "concentration–activity inhibition" dependence, with IC_{50} values of 52.3 ± 4.9 μM for midazolam and 46.1 ± 9.2 μM for testosterone. For metabolism-dependent inhibition, IC_{50} values were 36.6 ± 8.6 μM for midazolam and 28.3 ± 7.4 μM for testosterone. Given that the binding of propoxazepam to microsomal protein under the experimental conditions, which reflected those in the IC_{50} experiments, was low, no microsomal binding correction factor was applied to the reported IC_{50} values.

Conclusions. The highest predicted unbound C_{max} plasma concentration of propoxazepam, above which interactions can occur, is between 0.462 and 0.524 μM, or 462 and 524 nM. This corresponds to concentrations of 188 to 214 ng/mL (based on the molecular weight of propoxazepam, 414.73 g/mol). According to pharmacokinetic data, these concentrations are not achievable after a single oral administration. Further studies are required for multiple-dose administration.

Keywords: propoxazepam; CYP3A4; testosterone; midazolam; reversible inhibition; metabolism dependent inhibition.

Introduction

Cytochromes P450 (CYPs) are the heme-containing enzymes responsible for the oxidative metabolism of a wide variety of small molecule substrates [1]. The main and the most important metabolic reaction, catalysed by CYPs is the oxidation, where the hydroxylated reaction product (metabolite) forms. The catalytic activity of CYP is associated with redox partner proteins that transfer electrons from NADPH to the hemoprotein heme center [2]. More than 95% of the reported oxidations and reductions of all xenobiotics are mediated by CYPs [3].

Recent studies have demonstrated that, of the 110 commonly used drugs, 66% are metabolized by one or more CYP enzymes (such as 3A4, 2D6, 2C19, and 1A2 [4, 5]. CYPs are of critical importance due to the metabolism-mediated drug-drug interactions (DDI) and individual variability in drug metabolism [6]. Most drugs are deactivated by cytochrome P450 enzymes (CYPs), but some are bioactivated by these enzymes to pharmacologically active compounds. Additionally, many drugs may increase or decrease the activity of various CYPs due to their ability of binding to them. It is important to evaluate the potential inhibition of a new drug candidate for the most clinically relevant CYP450 enzymes.

One of the isoforms, CYP3A4, is the most important for drug metabolism. It is distributed in various tissues and is abundantly represented in the body's tissues, contributing to more than 70% of gastrointestinal CYP activity. The CYP3A4 enzyme is responsible for catalyzing approximately 33% of such reactions [7].

As many marketed drugs are metabolized by CYP3A4, drug-drug interactions (DDI) associated with it must be carefully studied and the high importance of mitigating risk with respect to CYP inhibition has been recognized by both regulatory agencies and by pharmaceutical companies.

Multiple probe substrates are often used for *in vitro* CYP3A4 DDI studies, including midazolam (the clinical standard) and testosterone. This is emphasized by the issuance of US Food and Drug Administration guidance documents in 2006 [8] and position papers by member companies of the Pharmaceutical Research and Manufacturers of America (PhRMA) [9] with a focus on the use of *in vitro* assessments of CYP inhibition and/or inactivation in the decision-making process regarding risk assessment and the initiation of clinical DDI trials.

Propoxazepam, as a promising analgesic drug, is undergoing clinical studies in Ukraine. Similar to gabapentinoid drugs (derivatives of the inhibitory neurotransmitter gamma-aminobuturyc acid, GABA), which are used in general medical practice in the treatment of neuropathic pain, propoxazepam also has an anticonvulsant effect [10–12], which is considered a predictor of analgesic action and thus explains the analgesic component in the pharmacological spectrum of compound. Data [13] suggest that the mechanism of propoxazepam's analgesic and anticonvulsant properties includes GABAergic and glycinergic systems, and the results of phase 1 clinical study demonstrated the appropriate safety profile with the pharmacokinetic data obtained for oral administration of 5 mg tablets in healthy subjects.

However, the inhibition of CYP3A4 activity by propoxazepam has not been studied. The aim of the study was to evaluate the effects of propoxazepam on CYP3A4 activity *in vitro* using testosterone and midazolam as classical markers of metabolic activity for CYP3A4 activity and human liver microsomes as a source of CYP3A4.

Materials and Methods

Propoxazepam and 3-hydroxy derivative (7-bromo-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2H-1,4-benzodiazepin-2-one, major metabolite) were synthesized according to the method [14]. The internal standard was supplied by SLC "Interchem" (Propoxazepam-D7 ($C_{18}H_9BrClD_7N_2O_2$)), purity ≥98.0%, MW = 414.73 g/mol). General-purpose reagents and solvents were of analytical grade (or a suitable alternative) and were obtained principally from VWR International Ltd, Rathburn Chemicals Ltd, Sigma Aldrich Chemical Company Ltd and Fisher Scientific UK Limited. Human liver microsomes (HLM) were obtained from Corning Ultra Pool HLM 150 (Lot 38292, Corning® UltraPool™ Microsome Hu Liver 150 Donor Pool, Merck, Germany).

Midazolam 1′-hydroxylation and testosterone 6β-hydroxylation were used as markers for CYP3A4-mediated activity. Stock solutions of propoxazepam were prepared in dimethylsylfoxide (DMSO) and diluted in the incubation mixtures such that the final organic solvent concentration attributable to the test substance was 0.5% (v/v). For the determination of the inhibitory potential of propoxazepam, HLM were incubated, in triplicate, with isoform-selective probe substrates, reduced nicotinamide adenine dinucleotide phosphate (NADPH) (2 mM) (Merck, Germany) and propoxazepam at concentrations between 0.1 and 100 μM. After equilibration, reactions were initiated by the addition of NADPH. Incubations were performed at 37 °C and terminated after the relevant incubation time by the addition of methanol, containing an internal standard (Propoxazepam-D7). The samples were then centrifuged for 5 minutes to sediment the precipitated proteins. Substrate concentrations approximated the K_m for CYP3A4. Vehicle samples contained an equivalent volume of the appropriate solvent in place of propoxazepam or the positive control [15].

Metabolism mediated inhibitory potential was investigated using a similar procedure, except that the HLM were pre-incubated for 30 minutes at 37 °C, in triplicate, with propoxazepam (0.1–100 μM) and NADPH prior to the addition of the CYP marker substrate (at a concentration which approximated to the K_m). The metabolism dependent inhibitor troleandomycin was used as positive controls for the effects on CYP3A4. The CYP enzyme activity for the pre-incubated samples in the presence of propoxazepam was compared to samples incubated in its absence.

The microsomal binding of propoxazepam was determined by equilibrium dialysis using the HTD equilibrium dialysis device (HTD dialysis). All incubations were carried out on an orbital shaker (200 rpm) placed within an incubator maintained at 37 °C and 5% CO_2/95% air. HLM were diluted with assay buffer solution to protein concentrations of 0.01, 0.05 and 1 mg/mL and spiked with propoxazepam at concentrations of 0.1, 10 and 100 μM. Triplicate spiked microsome samples were then dialyzed against assay buffer solution at 37 °C/5% CO_2 for 6 hours. Aliquots of the stock

spiked microsomes, together with samples from the protein and buffer solution chambers were then analyzed by using a qualified Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) method and the concentration of propoxazepam was calculated. The recovery of propoxazepam from the apparatus was also calculated.

The formation of the metabolites was quantified by LC MS/MS in Multiple Reaction Monitoring mode (MRM mode) using Electrospray ionization (ESI) as the ionization technique. Calibration standard working solutions were used to freshly prepare the calibration standards. An aliquot (50 μL (low range), 10 μL (high range) of the calibration standards, quality control (QC) samples and blanks was added to a 2 mL 96-well microplate. An equivalent volume of water was added to the reagent blank sample. Internal standard solution (Propoxazepam-D7; 10 ng/ml (low range) or 500 ng/ml (high range) in acetonitrile; 25 μL) was then added to the samples, calibration standards and QC samples. Acetonitrile (25 μL) was added to the blank samples. All samples were then vortex mixed (2000× g) for 10 minutes. Acetonitrile (150 μL (low range) or 400 μL (high range)) was added to all samples prior to vortex mixing (2000× g) for 10 minutes and centrifugation at 2000× g for approximately 5 minutes at 5 °C. Aliquots of the supernatant solutions (80 μL (low range) or 20 μL (high range)) were then transferred to a clean 96-well plate (automated liquid handling device Hamilton Microlab STAR). Acetonitrile (60 μL) was added to high range samples only. To all samples, 10 mM ammonium formate (aq.): formic acid (100:0.2 v/v) was added (120 μL). After centrifugation at 2000× g for approximately 5 minutes at 5 °C, the samples were stored at 2–8 °C (nominally 4 °C) prior to analysis by LC-MS/MS.

The activity of the enzyme, in the presence of various concentrations of propoxazepam, was expressed as a percentage of the appropriate control activity. Where the IC_{50} (the concentration at which the CYP probe substrate activity was reduced by 50%) could be determined, this was calculated by non-linear regression using validated SigmaPlot software (Version 12.5, Systat Software Inc).

Due to sub-optimal data fits, the data for the CYP3A4 reversible and metabolism-dependent inhibition assays were fitted to a 3-parameter equation without the background function:

$$y = \frac{Range}{1 + \left(\frac{x}{IC_{50}}\right)^{s}},$$

where *Range* is the maximum y range, i.e. control conversion rate (no inhibitor); s is slope factor; y is conversion rate of probe substrate to metabolite; x is propoxazepam concentration.

Assuming Enzyme competitive inhibition (K_i) can be estimated as follows:

$$IC_{50} = K_i\,(1 + S/K_m),$$

$$\text{If } [S] = K_m, \qquad \text{then } IC_{50} = 2K_i.$$

All substrate concentrations used in the current study approximated the K_m.

Results were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed fololwing the Shapiro–Wilk normality test, using unpaired Student's *t*-test. For *in vitro* determination of propoxazepam binding to human liver microsomes the comparisons were made relative to Group 1's data (two sample *t*-test). Also the IC_{50} values for reversible and metabolism dependent inhibition were compared using unpaired two sample *t*-test. Significance level was set at $p \le 0.05$.

Results

CYP3A4 activity inhibition. Propoxazepam at multiple (0 to 100 μM) concentrations consistently inhibited the activities of CYP3A4 (the Figure). To determine the possible effect of propoxazepam on reversible inhibition of 3A4, it was incubated with the microsomal fraction and corresponding substrates (midazolam or testosterone). It was found (Fig. 1a) that for both substrates, the "concentration–activity inhibition" dependence was similar, with IC_{50} values of 52.3 ± 4.9 μM for midazolam and 46.1 ± 9.2 μM for testosterone (Table 1).

For metabolism-dependent inhibition propoxazepam was previously incubated (30 minures) with microsomal fraction prior to substrates addition (Fig. 1b), resulting in IC_{50} values of 36.6 ± 8.6 and 28.3 ± 7.4 μM for midazolam and testosterone as substrates, respectively (Table 1). For both variants the used positive controls (ketoconazole, 0.2 μM, for reversible inhibition and troleandomycin, 50 μM for metabolism dependent inhibition) demonstrated the expected 3A4 activity inhibition (to 9.6% and 7.9%, respectively, in compare to the control).

The effects of propoxazepam on the microsomal CYP3A4-mediated enzyme activity and calculated IC_{50} values are presented in Table 1.

Microsomal Binding. The results of the microsomal binding experiment are summarized in Table 2. Post-dialysis recoveries are presented in Table 3,

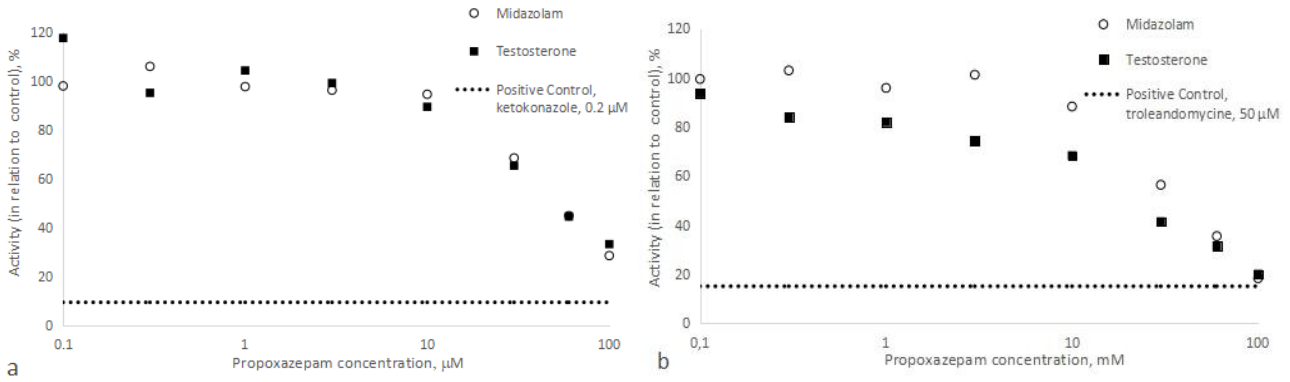

Figure: Effect of propoxazepam on CYP3A4-mediated midazolam 1′-hydroxylation and testosterone 6β-hydroxylation in human liver microsomes: (a) reversible inhibition and (b) metabolism-dependent inhibition

Table 1: The ability of propoxazepam to inhibit CYPs activities *in vitro* in human liver microsomes (M ± m, $n = 3$ for each experimental point)

Substrare	IC_{50} (μM)		
	Reversible inhibition	Metabolism-dependent	IC_{50} shift
Midazolam	52.3 ± 4.9	36.6 ± 8.6	1.43
Testosterone	46.1 ± 9.2	28.3 ± 7.4	1.63

Table 2: *In vitro* determination of binding of propoxazepam (0.1, 10, and 100 μM) following dialysis of spiked human liver microsomes for 6 hours

Nominal concentration (μM)	HLM (mg/ml)	Actual concentration (μM)	Donor concentration (μM)	Acceptor concentration, (μM)	Fraction bound (%)	Fraction unbound (%)	Fraction unbound (mean value)
0.1		0.045 ± 0.002	0.018 ± 0.002	0.019 ± 0.001	–6.2 ± 2.3	106.1 ± 7.1	
10	0.01	8.2 ± 0.3	3.3 ± 0.2	3.6 ± 0.6	–7.2 ± 2.8	107.4 ± 3.5	103.6 ± 3.2
100		85.9 ± 2.5	36.8 ± 3.4	35.7 ± 2.5	2.7 ± 2.6	97.3 ± 1.4	
0.1		0.046 ± 0.001	0.021 ± 0.001	0.018 ± 0.001	6.8 ± 1.9	93.2 ± 2.3	
10	0.05	8.6 ± 0.3	4.2 ± 0.6	3.7 ± 0.6	11.7 ± 3.6	88.3 ± 3.5	88.5 ± 2.7*
100		85.5 ± 1.1	39.5 ± 5.1	32.9 ± 2.2	16.1 ± 4.7	83.9 ± 3.3	
0.1		0.049 ± 0.001	0.035 ± 0.009	0.011 ± 0.002	70.3 ± 4.5	29.7 ± 2.6	
10	1	8.2 ± 0.2	6.5 ± 0.3	1.6 ± 0.3	75.1 ± 6.4	24.9 ± 3.5	30.3 ± 3.3**
100		98.8 ± 4.1	65.1 ± 11.7	22.3 ± 5.7	63.7 ± 16.5	36.3 ± 9.5	

Notes. HLM – human liver microsomes, * – statistically significant at $p \le 0.05$ (compared to group 1), ** – statistically significant at $p \le 0.01$ (compared to group 1).

Table 3: Microsomal binding of propoxazepam (0.1, 10 and 100 μM): post-dialysis recoveries

Nominal concentration (μM)	HLM (mg/mL)	Recovery (%)
0.1	0.01	83.0 ± 6.6
10	–	84.1 ± 5.0
100	–	84.4 ± 6.8
0.1	0.05	82.1 ± 1.7

Table 4: Estimated K_i values based on an assumed competitive mechanism of direct inhibition

Substrate	IC_{50} (μM)	Estimated K_i (μM)	Unbound plasma concentration where $R1 \geq 1.02^{\#}$ or $0.02^{\#\#}$ (μM)
Midazolam	52.3	26.2	0.524
Testosterone	46.1	23.1	0.462

Notes. # – FDA 2020 [25], ## – EMA 2013 [24].

showing that microsomal binding was not notably dependent on propoxazepam concentration but was dependent on microsomal protein concentration.

The obtained data for reversible inhibition were used to estimate the ranges of substrate and inhibitor concentrations for the inhibition of CYP3A4 isoform by propoxazepam in HLMs, for precise estimation of inhibition constants (K_i values, Table 4).

Discussion

In this experiment, human liver microsomes (HLM) were utilized to estimate potential drug-drug interactions involving the investigated substance and recommended substrates. This method is particularly relevant, as it provides a reliable *in vitro* model for assessing metabolic pathways mediated by cytochrome P450 enzymes, which play a crucial role in drug metabolism. By using liver microsomes, it is possible to mimic the enzymatic activity that occurs *in vivo*, allowing for the prediction of how the investigated compound might interact with other drugs in a clinical setting. However, there are certain limitations to this approach. For instance, while liver microsomes offer insights into CYP-mediated metabolism, they do not account for the complex physiological factors present in a living organism, such as regional blood flow, protein binding, and the influence of transporters. Moreover, variations in enzyme expression between individuals can lead to discrepancies in predicted interactions. Despite these restrictions, the use of HLM remains a valuable preliminary step in drug development, helping to inform further studies and decision-making regarding clinical trials and is recommended by Regulatory agencies, as the first step in DDI estimation [16, 17].

For investigational new drugs the evaluation of drug–drug interactions (DDI) is one of the important stages and CYP3A4, which is involved in about 50% of drug metabolism is the pivotal when determining drug's DDI potential. Previous studies have shown that many 1,4-benzodiazepines, such as midazolam, triazolam, alprazolam, and diazepam, are primarily metabolized by CYP3A4, indicating a common metabolic pathway for this class of compounds. For example, midazolam and triazolam are well-known to undergo extensive hepatic metabolism via CYP3A4, resulting in their relatively short half-lives and potential drug-drug interactions. Given the structural similarities, it is likely that propoxazepam as 1.4-benzodiazepine derivative may be metabolized in a similar manner, which could affect its pharmacokinetics and clearance [18]. Thus, for new analgesic, propoxazepam, LC-MS/MS-based CYP3A4 inhibition assay using selective substrates (midazolam and testosterone) was undertaken.

When suspecting CYP3A4 enzyme inhibition it should be kept in mind that there are significantly different types of inhibition and, therefore, different clinical implications [19]. Inhibition can be caused by the drug directly (Propoxazepam), or it can be caused by the metabolite that is produced by the CYP catalytic cycle. Inhibition caused by the propoxazepam directly can be classified as direct or time dependent. An inhibition caused by the metabolite can be classified as mechanism dependent (reversible or irreversible) or quasi-irreversible. According to previous studies [20], after 4-hour incubation with human hepatocytes, propoxazepam accounted for 96.0% of the profile. The most abundant metabolite formed was oxidized propoxazepam (3-hydroxyderivative), which accounted for approximately 2.5% of the total peak response in the 4-hour sample.

Testosterone is the endogenous CYP3A4 substrate which in the presence of NADPH undergoes metabolism with the formation of hydroxylation products (6β-, 2β-), but mostly the 6β-hydroxyderivative, with C_{50} 26.1 ± 6.4 μM [21]. On the other hand, midazolam, as the sensitive marker substrate for 3A4 drug metabolism, was also suggested [22], since at low substrate concentrations it gives the 1-OH derivative, with 4-OH derivative formation only at high midazolam concentrations [23] and both these substrates are used for *in vitro* DDI probes for new drugs investigation, according to EMA Guideline (Guideline on the investigation of drug interactions, 2012) [16] and FDA [17]. In this study propoxazepam showed similar concentration-dependent 3A4 activity inhibition for both mida-

zolam and testosterone as substrates (Fig. 1a) with the close IC_{50} values (52.3 ± 4.9 μM for midazolam and 46.1 ± 9.2 μM for testosterone) in the reversible inhibition experiments. As propoxazepam has a structure, similar to midazolam, one can assume that the competitive inhibition of the unchanged drug can occur with the structures which either have the structural similarity to 1.4-benzodiazepine structure or stereochemically match.

In the metabolism dependent experiment the possible influence of propoxazepam metabolites on 3A4 activity was estimated by means of a previous incubation of the microsomes with propoxazepam for 30 min before adding the corresponding substrate (see Fig. 1). After the mentioned period the activity of 3A4 was measured by adding the specific substrate (either midazolam or testosterone) and it was noted that despite the structure similarity for midazolam, as substrate the determined IC_{50} 36.6 ± 8.6 μM was quite higher than for testosterone (28.3 ± 7.4 μM) (see Table 1). Taking as an indication of the possible inhibition by metabolism-dependent mechanism a ≥2-fold shift in the IC_{50} value (ratio of reversible and metabolism-dependent) it can be seen that on both midazolam (shift of 1.43) and testosterone (1.63) there is a certain possibility of 3A4 activity inhibition in the presence of propoxazepam.

Microsomal binding of propoxazepam was additionally studied, as one of the possible sources of concentration influencing factor. As the local saturation of microsomes (proteins and lipids) with propoxazepam can determine its ratio between solution and microsomes, the experiment was designed to estimate the microsomal binding depending on propoxazepam and HLP concentrations. It was shown that in the range of the concentrations used (0.1−100 μM), propoxazepam nonspecific binding was not influenced by the compound concentration (no statistically significant difference). In contrast, HML concentration an increase in HLM concentration led to a statistically significant ($p \geq 0.05$ for HMP concentration 0.05 mg/mL and $p \geq 0.01$ for HMP concentration 1.0 mg/mL respectively) difference in value of the unbound fraction (Table 2). Notably, the absence of an influence of the propoxazepam and HML on the nonspecific binding (Mean free fractions at microsomal protein concentrations of 0.01, 0.05 and 1 mg/mL were $103 \pm 3{,}2\%$, $88.5 \pm 2{,}7\%$ and $30.3 \pm 3{,}3\%$, respectively, over the propoxazepam concentration range used, Table 3), and given the low binding of propoxazepam to microsomal protein, when incubated under conditions reflecting those in the IC_{50}, no microsomal binding correction factor was applied to the reported IC_{50} values.

Prediction of a potential DDI with CYP3A4 is challenging due to a number of *in vitro* and *in vivo* factors. The complexity of the *in vitro* kinetics observed for some of the CYP3A4 probes [24], CYP3A inter-individual variability in both liver and small intestine (including the variable contribution of polymorphically expressed CYP3A5) [25] confound the straightforward prediction of a DDI.

For a reversible (direct) inhibitor, the likelihood of a clinically relevant drug interaction can be predicted using a basic model with K_i values which can be estimated from IC_{50} values, or experimentally determined [26]. The obtained data were then used to simulate the appropriate ranges of substrate and inhibitor concentrations for the inhibition of CYP3A4 isoform by propoxazepam in HLMs for the precise estimation of inhibition constants (K_i values, see Table 4).

So, the calculated values of 3A4 K_i for midazolam and testosterone as substrates can be estimated as 26.2 and 23.1 μM correspondingly.

The 2013 European Medicines Agency (EMA) guidance [24] suggested that "an *in vivo* drug-drug interaction study with a sensitive probe substrate is recommended when $[I]/K_i \geq 0.02$, where [I] is the unbound mean C_{max} value obtained during treatment with the highest recommended dose". Thus, the highest predicted unbound C_{max} plasma concentration of propoxazepam, above which the interaction can occur, is between 0.462 and 0.524 μM, or 462 and 524 nM, this, when considering the molecular weight propoxazepam 414.73 g/mol) corresponds to concentrations ranging from 188 to 214 ng/ml.

According to our data [27], the unbound propoxazepam fraction in human plasma is 1.96%, therefore, the total concentration at which inhibition is predicted to occur, is 9592−10918 ng/ml. Pharmacokinetics study result show that "the maximum propoxazepam concentration (22.276 ng/ml) was reached in blood by 4 hours after oral administration on healthy volunteers" [28], that was much lower, than the estimated prognosed inhibition levels. Based on this it can be concluded that propoxazepam is not expected to be the CYP 3A4 inhibitor *in vivo*.

Conclusions

Propoxazepam is predicted to cause clinically relevant drug interactions with co-administrated CYP3A4 substrates at unbound plasma C_{max} concentrations of ≥0.524 μM (approx. 214 ng/mL) and

0.462 μM (approx. 188 ng/mL). A 30 minutes pre-incubation of propoxazepam with microsomes and NADPH prior to substrate addition did not result in a sigificant change in these values, which suggested that the inhibition mechanism was metabolism dependent and rather than reversible direct inhibition. According to our pharmacokinetics data at least after single oral administration these concentrations are not reachable. Further studies are required to evaluate potential interactions during multiple course administration.

Interests disclosure

A.S. Reder reported to be an employee of SLC "Interchem", the financial sponsor of this study. The authors declare that this financial support did not influence the study design, data collection, analysis, interpretation, or manuscript writing.

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ІНГІБУВАННЯ АКТИВНОСТІ ЦИТОХРОМУ P450 ПРОПОКСАЗЕПАМОМ: ОЦІНКА БЕЗПЕКИ ПОТЕНЦІЙНОЇ ЛІКАРСЬКОЇ ВЗАЄМОДІЇ

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Проблематика. Пропоксазепам є новим аналгетиком групи бензодіазепінів, хімічно 7-бром-5-(о-хлорфеніл)-3-пропілокси-1,2-дигідро-3Н-1,4-бензодіазепін-2-он. Пропоксазепам розглядається як можливий субстрат системи CYP, тому його вплив на активність ферменту CYP3A4 було досліджено *in vitro* з використанням мікросом печінки людини.
Мета. Оцінка впливу пропоксазепаму на активність CYP3A4 *in vitro* з використанням тестостерону та мідазоламу як маркерів метаболічної активності CYP3A4 у мікросомах печінки людини.
Методика реалізації. Мідазолам (реакція 1′-гідроксилювання) і тестостерон (реакція 6β-гідроксилювання) використовувалися як маркери активності, опосередкованої CYP3A4. Як позитивний контроль використовували кетоконазол 0,2 μM (для оборотного інгібування) і тролеандоміцин, 50 μM (для інгібування, залежного від метаболізму). В умовах оборотного інгібування пропоксазепам додавали разом із відповідним субстратом і кофактором (НАДФН), тоді як у залежному від метаболізму стані його інкубували 30 хв із мікросомами та кофактором перед додаванням субстрату.
Результати. Пропоксазепам у кількох (від 0 до 100 μM) концентраціях послідовно інгібував активність CYP3A4, для обох субстратів залежність “концентрація–інгібування активності” подібна з IC_{50} 52,3 ± 4,9 μM для мідазоламу та 46,1 ± 9,2 μM для тестостерону. Для залежного від метаболізму інгібування IC_{50} становила 36,6 ± 8,6 μM і 28,3 ± 7,4 μM для мідазоламу та тестостерону як субстратів відповідно. З огляду на те, що зв’язування пропоксазепаму з мікросомальним білком під час інкубації в умовах, які відображали умови в експериментах IC_{50}, було низьким, до повідомлених значень IC_{50} не було застосовано поправковий коефіцієнт зв’язування з мікросомами.
Висновки. Найвища прогнозована концентрація C_{max} незв’язаного пропоксазепаму в плазмі, вище якої може відбутися взаємодія, становить від 0,462 до 0,524 μM, або 462 і 524 нМ, що (з молекулярною масою пропоксазепаму 414,73 г/моль) дає від 188 до 214 нг/мл. Згідно з даними фармакокінетики, принаймні після одноразового перорального прийому ці концентрації не досягаються. Для проведення багаторазового курсу необхідні додаткові дослідження.

Ключові слова: пропоксазепам; CYP3A4; тестостерон; мідазолам; оборотне інгібування; залежне від метаболізму інгібування.