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ORIGINAL ARTICLE

A Phase I study evaluating the pharmacokinetic profile of a novel oral analgesic propoxazepam

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Abstract

Introduction and Objective. Propoxazepam, 7-bromo-5 - (o-chlorophenyl)-3-propoxy – 1,2-dihydro – 3H-1,4- benzodiazepin-2-one, in the models of nociceptive and neuropathic pain showed significant analgesic activity. The aim of the study was to investigate the pharmacokinetics of propoxazepam and its metabolites after a single oral dose in healthy volunteers.

Materials and method. 12 volunteers were orally dosed with 5 mg propoxazepam under fasting conditions. Blood samples were collected up to 72 hours after administration and propxazepam extracted with liquid-phase extraction and analyzed with high-performance liquid chromatography–tandem mass spectrometry.

Results. Maximum propoxazepam concentration (22.276 ng/mL) was reached in blood by 4 hours after administration. It had a large volume of distribution (~6.3 L/kg), the elimination half-life 30.11 hours, MRT 37.77 hours, common clearance – 9062.929 mL/hour. Both propoxazepam and its metabolites (3-hydroxy derivative and glucuronide) were detected in the urine of volunteers. The urinary excretion rate of propoxazepam is proportional to its concentration in plasma. Only a small amount of unchanged propoxazepam was excreted with urine – 0.062 % of the administered dose. Renal clearance – 6.46 mL/hour.

Conclusions. A single dose (5 mg) of Propoxazepam administered orally showed good tolerability, pharmacokinetically characterized by rapid absorption, slow elimination and low quantities of unchanged parent urinary excreted. The oxidized metabolite (3-hydroxypropoxazepam) and its glucuronide were excreted with urin, a total of up to 10.5% of the administered dose, which indicates a high degree of metabolism and possible hepatointestinal circulation.

Key words

metabolism, pharmacokinetics, excretion, propoxazepam

INTRODUCTION

The gate control theory of pain [1] proposes that inhibitory neurons in the superficial dorsal horn of the spinal cord control the relay of nociceptive signals from the periphery to higher areas of the central nervous system. The key role of inhibitory γ-aminobuturic acid – ergic (GABAergic) – and glycinergic neurons in this process has recently been demonstrated in several reports indicating that a loss of inhibitory neurotransmission underlies several forms of chronic pain [2, 3]. Classical benzodiazepines (BDZ), the allosteric modulators of inhibitory type A GABA receptors (GABAA receptors), are commonly used for their sedative, anxiolytic, and anti-convulsant effects, largely lack overt analgesic efficacy in humans when administered systemically. Therefore, they have rarely been considered as medications for analgesic treatment.

Our knowledge of the certain subtype selective BDZ pharmacology revived the drug research towards GABAA receptors modulators. Selectivity is achieved by subtype-dependent binding affinity or efficacy. The latter results from full or partial agonism at one subtype but full or partial antagonism, weaker agonism (or negative agonism) at other subtypes. Among the most advanced fields is the search for non-sedative anxiolytic drugs, targeting the subunit α2 while sparing the α1-subtype mediated effects, including sedation, impairment of motor coordination, attention and memory deficits, and promotion of abuse [4].

Subtype-selectivity of GABAA receptors modulators is of further interest in pharmacological intervention in pain [5]. Subtype-selective compounds targeting the α2 and/or α3 subunit of the GABAA receptors produce antihyperalgesia in mice and rats without sedation and without tolerance induction [6]. The analgesic effects of benzodiazepine agent midazolam, based on influencing spinal GABAA receptors on its subunits α2, α3 and α5, have been shown both in animal models and in humans [7, 8]. These findings open new perspectives for a more selective targeting of pain pathways with GABAergic drugs.

Propoxazepam is considered a promising drug and is undergoing clinical studies in Ukraine. Similar to gabapentinoid drugs (derivatives of the inhibitory neurotransmitter gamma – gamma-aminobuturic acid, GABA), which are used in general medical practice in the treatment of neuropathic pain [9], propoxazepam also has an anti-convulsant effect [10–12], which is considered as a predictor of analgesic action and thus explains the analgesic component in the pharmacological spectrum of compound. Data [10–13] suggest that the mechanism of propoxazepam

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analgesic and anti-convulsant properties includes GABAergic and glycinergic systems.

This phase 1 clinical study aimed to characterize the safety, pharmacokinetic profile and metabolism of an investigational drug – propoxazepam in 5 mg tablets in healthy subjects.

MATERIALS AND METHOD

Study design, participants and drug. The single centre, open-label clinical trial was conducted to evaluate the safety and pharmacokinetic profiles of propoxazepam in 12 healthy male participants aged 18–50 years under fasting conditions. All participants provided written informed consent and were screened for inclusion (healthy male non-smokers, 18–50 y.o., body mass index (BMI) ≥18.5 kg/m^2 and ≤30 kg/m^2), and exclusion (known hypersensitivity to benzodiazepines or other allergy, gastrointestinal disorders, HbsAg/HCV/HIV/SARS-CoV-2, or urine drugs positive test, as well as positive breath alcohol test, administration of any medicines, plant medicines, dietary supplements, vitamins etc). 14 days prior to the study. During the study, all concomitant medications administration and xanthine-containing beverages were disallowed. The study was conducted following an ethical approval from the official Ukrainian regulatory body – the State Expert Centre of the Ministry of Health of Ukraine in accordance with the current legislation [14–17].

The studied drug, propoxazepam 5 mg tablets provided by SLC 'INTERCHEM' (Batch No. 600719, expiry date: 02.2022), was administered (200 ml) as one dose under fasting conditions 30 min. before breakfast with water.

Safety assessments. All participants who received a dose 5 mg of propoxazepam were included in the safety population. Assessments included physical examinations, recording of vital signs, electrocardiography, clinical laboratory tests (biochemistry, haematology, HbsAg/HCV/HIV screening), urinalysis and urine drug, and alcohol breath tests. Each participant was questioned regarding adverse events. The nature, time, duration, and severity of all adverse events were recorded (severe adverse event is that which can cause death, is life threatening, requires hospitalization; non-serious adverse events are all other medication-connected events – dizziness, headache, discoordination, ataxia, muscle weakness graded according to the patient`s severity state). The relationship between the adverse event and the formulation was categorized by a responsible investigator as likely related, unlikely related, or unrelated.

Pharmacokinetic evaluation. In each period, blood samples (6 mL at each post dosing time point, in total no more than 207 mL throughout the study) were collected via the cannulated cubital vein at pre-dose (within 0.5 h before dosing, double volume, 12 mL) and 0.25; 0.5; 0.75; 1; 1.5; 2; 2.5; 3; 4; 5; 6; 8; 10; 12; 16; 20; 24; 30; 36; 46, 58 and 72 h following dose administration (a total of 23 blood samples from each volunteer). The blood serum samples were analyzed for the parent substance, using a validated LCMS method.

Collection and preparation of blood and urine samples. Blood samples were collected in vacutainer tubes. After collection, containers with blood remained at room temperature (20±2°C) for 20 min for blood clotting, and then centrifuged in a refrigerator centrifuge at 1,800 g for 10 min at +4°C to obtain serum. Each of 2 aliquots (no less than 1.2 mL) was placed in the separate pre-labeled primary cryocontainers (tubes). Before use, the frozen samples were stored at -20 °C.

For urine collections the volunteers emptied their bladders, regardless the need (at 7.40–7.45 AM, time point zero). After drug administration the urine sampling was made 'on demand', pooling urine portions at each specific interval. The urine samples of 12 volunteers were obtained after single drug administration according to the schedule: at time 0 (before administration) and during 0–4 hours, 4–8 hours, 8–12 hours, 12–18 hours, 18–24 hours, 24–36 hours, 36–48 hours, 48–60 hours, and 60–72 hours after administration. 5 mL of urine sample were placed in pre-labeled disposable test tubes and frozen at -70°C.

Analytical methods. Samples of serum and urine were tested for interference with peaks of propoxazepam and propoxazepam-D7 (internal standard). The samples which passed the test were used for preparing blanks, samples for chromatographic system suitability, calibration standards and QC-samples.

Quantitative determination of propoxazepam in human serum and urine was performed by a validated bioanalytical method, using chromatographic system 'Agilent 1260' with MS/MS detecting 'Agilent 6420 Triple Quad'. Preliminary sample preparation included liquid-liquid extraction with a mixture of dichloromethane – hexane (1:1), followed by evaporation of the organic layer to dry precipitate and its dissolution with 70% methanol.

Analyte standard. Working standard sample (WSS) of propoxazepam (SLC 'INTERCHEM', Batch No. 65.12010619), certified value of the main substance – 99.62%. Storage conditions: in a tightly closed container, in a dark place at temperature 2–8°C. Internal standard: propoxazepam – D7, SLC 'INTERCHEM' (Batch No. 010620), certified value of the main substance – 99.41%, isotope purity – 98.0%. Storage conditions: in a tightly closed container, in a dark place at temperature 2–8°C.

Determination of metabolites content. Test urine samples (5 ml) were placed in centrifuge tubes, and lipophilic components extracted with dichloromethane (3×3 mL) with intermediate centrifugation of the resulting emulsion to separate it more efficiently. The combined extracts were evaporated under warm air.

To the aqueous phase 2.0 mL of acetic buffer (pH 3.5, 0.1 M) and 30 mg of β-glucuronidase were added, and after mixturing, incubated during 24 hours at 37.0 ± 0.5 °C. The formed lipophilic products were then extracted with dichloromethane (3×3 mL) with further extragent evaporation, as mentioned above.

To the obtained dry residues, 1.0 ml of mobile phase B was added and chromatographed under the following conditions: a stainless steel column 100×4.6 mm, filled with octadecylsilyl silica gel for chromatography with a particle size of 3.5 μm; precolumn of the corresponding configuration. Mobile phase: phase A – acetonitrile-water-formic acid (400:600:2), phase B – acetonitrile (in gradient supply); injection volume 50 μL. The developed analytical method of corresponding metabolites determination had a wide linearity range

with LLOQ 10 ng and ULOQ 1000 ng, which is equal to 2–200 ng/mL of the substance in the sample (linearity R2 = 0.99882, accuracy – 1.62 % to +2.14%, precicion <1.4%; recovery 97.2–108.5%). Samples with a quantity below LLOQ were analysed with a higher injection volume (100 µL) and, if the quantity of the substance was still below LLOQ, assumed to have '0' content. No samples were above ULOQ.

Pharmacokinetic analysis. Non-compartmental analysis of the pharmacokinetic data was performed using the Phoenix WinNonLin package (Certara Inc., New Jersey, USA). The following pharmacokinetic parameters were estimated: maximum observed plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration-time curve from time 0 to the last measured concentration (AUC_{0-8}), and from time 0 to time infinity (AUC_{inf}) estimated using the linear-up-log down trapezoidal method, apparent terminal elimination rate constant (ke) as determined by linear regression of the terminal points of the log-linear concentration-time curve, and the apparent terminal half-life (t1/2) calculated as 0.693/ke.

Statistical analysis. The results of the quantification of the concentration of propoxazepam in the serum and urine of volunteers, as well as pharmacokinetic data were subject to statistical analysis (Phoenix WinNonLin, Certara Inc., New Jersey, USA). The arithmetic mean (Mean), geometric mean (GM), standard deviation (SD), standard error of the mean (SE), coefficient of variation (CV%), median, minimum, and maximum values were calculated.

RESULTS

Safety and tolerability. The drug was well tolerated and no serious adverse events were reported during the study after single administration of a 5 mg dose of propoxazepam.

Pharmacokinetic Parameters. The propoxazepam plasma concentration-time profiles are shown in Figure 1 and pharmacokinetic parameters are summarized in Table 1. Concentration-time profile of propoxazepam in serum after oral administration was a maximum of 3.5–4 hours with the exponential concentration decrease (first order kinetics) (Fig. 1). Pharmacokinetic parameters were calculated from the mean plasma concentrations of propoxazepam (Tab. 1).

The maximum concentration of propoxazepam in the serum was observed after 4 h (range 0.75–6.00 h) after administration – 22.28 (15.708–27.048) ng/ml. It should be noted that propoxazepam has a high value of both total (378974.77 ± 75830.89 mL) and specific (about 6.3 L/kg) volume of distribution. The half-life from the serum (half-elimination time) is quite long 30.11 (20.63 – 49.39) h). Due to long-term elimination from the serum, the mean residence time is 37.77 h. Total clearance after oral administration – 9062.93 mL/h.

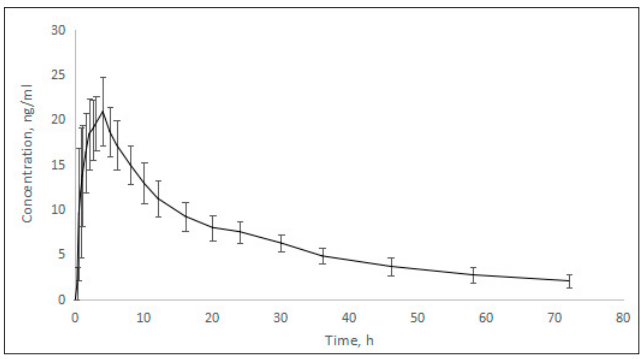


Figure 1. Mean plasma concentration vs. time curve for propoxazepam (linear plot, mean ± SD)

Table 1. Summary of pharmacokinetic parameters of propoxazepam

Parameters (units)	Mean ± SD (CV %)
AUC_{0-t}, ng×hour/mL	479.8±76.3 (15.90%)
$AUC_{0-\infty}$, ng×hour/mL	576.3±131.9 (22.88%)
C_{max}, ng/mL	22.28±3.45 (15.48%)
T_{max}, hours	3.5±1.4 (39.30%) 4.00 (0.75÷6.00)a
t1/2, hours	30.1±8.8 (29.14%)
MRT, hour	37.77±10.44 (27.48%)
Kel, $hour^{-1}$	0.025±0.006 (23.58%)
Cl_T, mL/hour	9062.93±1907.02 (21.04%)
Vz_F_obs, mL Vz_F_spec, L/kg	378974.77±75830.89 (20.01%) ~ 6.3
K01_HL, hour[b]	0.809±0.405 (50.01%)

a – median; in brackets – minimum and maximum values; b – using the one-compartment model with absorption; CV – coefficient of variation; AUC0-t – area under the concentration-time curve from 0 to the last determined concentration (≥LLOQ); AUC0-∞ – area under the concentration-time curve from 0 extrapolated to infinite time; Cmax – the first maximum concentration; Tmax – time to peak maximum concentration; t1/2 – elimination half-life; MRT – mean residence time; Ke – elimination constant; CIT – total clearance after oral administration; Vz_F_obs – observed volume of distribution; Vz_F_spec – specific volume of distribution; K01_HL – calculated half-absorption time

The values of the coefficients of variation (CV, %) show that the pharmacokinetic parameters of the degree of absorption, first maximum concentration, C_{max}; area under the concentration-time curve from 0 to the last determined concentration, AUC0-t and area under the concentration-time curve from 0 extrapolated to infinite time, $AUC_{0-\infty}$ and excretion (mean residence time, MRT; elimination half-life, t1/2, elimination constant, Kel; total clearance after oral administration, Cl_T had a low variability of <30%, while absorption rate parameters (time to peak maximum concentration, T_{max}; calculated half-absorption time, K01_HL), had a significant range of inter-subject variation.

Propoxazepam and its metabolites urine concentration-time profiles. Urine is a conventional specimen for monitoring parent drug and its metabolites. The target metabolite of propoxazepam, which was determined in the urine of volunteers, was 3-hydroxy derivative (3-hydroxypropoxazepam). The analytical method used in this study has proven to be sensitive and accurate for the analysis of propoxazepam and its metabolites in urine. The lower limit of quantitative determination LLOQ (0.05 ng/mL) is useful for quantifying the lower levels of propoxazepam, presented in urine during pharmacokinetic investigation (Fig. 2). For 3-hydroxypropoxazepam (and consequently, glucuronide which is detected through 3-hydroxypropoxazepam), the lower limit of detection was 10 ng, which corresponds to LLOQ 2 ng/ml in the initial samples.

After propoxazepam administration at single dose of 5 mg, a small amount of unchanged compound was detected in the urine at all time intervals (Fig. 2, A), with a maximum elimination rate of 4–8 hours and a gradual exponential

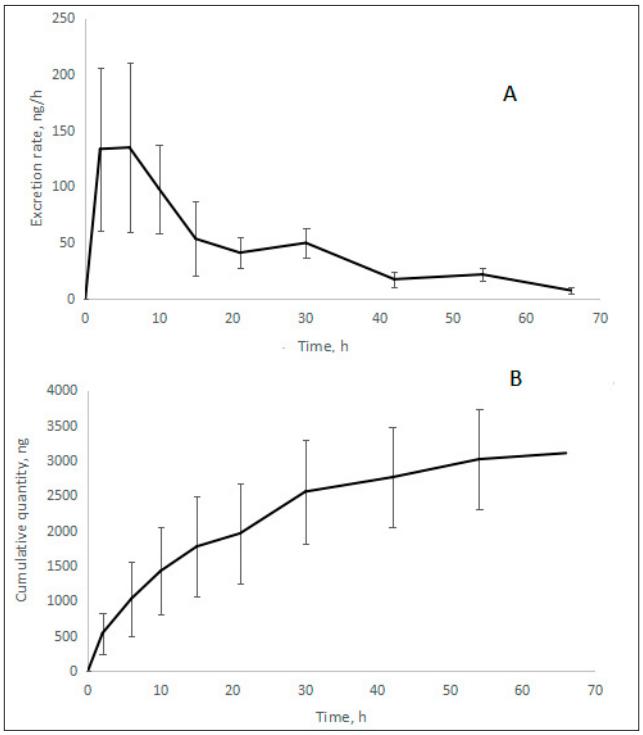


Figure 2. Concentration-time curves (Mean ± SD) of propoxazepam in urine (A) and cumulative urinary excretion (B)

Table 2. Pharmacokinetic parameters of propoxazepam in urine

Pharmacokinetic parameter	Arithmetic mean ± standard deviation SD (CV)
R_{max}, ng/hour	163.14±64.46 (39.52%)
Tmax_Rate, hour	4.67±3.11 (66.73%) 4.00 (2.00÷10.00)a
Kel (u), hour-1	0.0453±0.0092 (20.28%)
t1/2 (u), hour	15.9±3.2 (20.28%)
$AURC_{0-t}$, ng×hour/mL	2999.9±633.01 (21.10%)
$AURC_{0-\infty}$, ng×hour/mL	3177.43±623.01 (19.61%)
Ae(0-t), ng	3099.84±697.23 (22.49%)
Ae(%), %	0.062±0.014 (22.49%)
ClR, mL/hour	6.46

a – median; in brackets – minimum and maximum values; CV – coefficient of variation; R_{max} – Maximum rate of urinary excretion; Tmax_Rate – the time to reach maximum excretion rate; Kel (u) – Elimination rate constant with urine; t1/2 (u) – urinary excretion half-life; $AURC_{0-t}$ – area under the "urinary excretion rate- time" curve from 0 to the last determined concentration (≥LLOQ); $AURC_{0-\infty}$ – area under the "urinary excretion rate- time" curve from 0 to infinite time; Ae(0-t) – common (cumulative) urinary excretion from time 0 to the last determined point; Ae(%) – cumulative excretion in % of the administered dose; ClR – renal clearance

decrease. Up to 72 hours cumulative excretion reached a maximum value of ~ 3,000 ng (Fig. 2B), which is about 0.06% of the administered dose.

Table 2 represents the mean values (± SD and CV) of the pharmacokinetic parameters of propoxazepam in urine after single dose (5 mg) administration.

These parameters indicate that the maximum urinary excretion of propoxazepam is 163.14 (90.10 – 326.02) ng/ml and is observed after 4 h (median T_{max} = 4 h; range 2.00 – 10.00 h) after dosage. The elimination half-life from urine is 15.9 (10.77 – 21.40) hours. Cumulative excretion, on average, 3099.84 (2149.59 – 4512.80) ng.

In general, a very small amount of propoxazepam was excreted unchanged in the urine: only 0.062% of the administered dose (5 mg). Renal clearance averages 6.46 ml/h, indicating that the compound is eliminated by other routes, both metabolic and probably faecal.

After selection of appropriate chromatographic conditions in the mass spectra of samples after urine extraction (volunteers 302, 307, 309, 310), peaks were found which corresponded to one of the expected metabolites – 3-hydroxypropoxazepam. No other expected metabolites (hydroxylated by alkoxy group, aromatic ring) were detected under these conditions of the analysis (absence of characteristic chlorobromine isotopic cleavage of peaks in mass spectra).

To determine the metabolites presence, the urine samples from 4 volunteers with the highest propoxazepam concentrations were selected. The amount of detected 3-hydroxyphenazepam (based on the total amount excreted, corrected to urine volume) exceeded the content of propoxazepam 3–15 times for different samples (Tab. 3). Previous model experiments have shown that 3 extractions with dichloromethane (3:5 – 1:2 by volume, extragent: sample) leads to almost complete (> 97%) extraction of 3-hydroxypropoxazepam from the sample, therefore providing the possibility to further quantify glucuronide 3-hydroxypropoxazepam after hydrolysis.

Significant amounts of 3-hydroxypropoxazepam were detected in dichloromethane extracts of urine after incubation with β-glucuronidase (30 mg + addition of acetate buffer, pH 3.5). The amount of glucuronide is almost an order of magnitude greater than free 3-hydroxypropoxazepam (Tab. 3).

To estimate the total amount of compounds excreted in the urine, cumulative excretion (total amount of substance excreted by time t) was determined, as well as the proportion of excreted products from the administered dose was calculated (Tab. 4).

Table 3. The amount (in μg, calculated as the parent compound and its metabolites) of propoxazepam (PPZ), 3-hydroxypropoxazepam (3-OH-PPZ) and its glucuronide (GLUC-3-OH-PPZ) excreted in the urine of volunteers 302, 307, 309 and 310

Time, h	PPZ, μg				3-OH-PPZ, μg				GLUC-3-OH-PPZ, μg			
	302	307	309	310	302	307	309	310	302	307	309	310
0-4	0.55	0.47	0.41	0.64	5.48	3.49	7.89	5.75	23.91	16.82	6.29	8.47
4-8	0.38	0.58	0.31	0.59	1.32	2.48	0.70	8.71	29.66	23.31	6.60	25.00
8-12	0.39	0.46	0.57	0.35	2.90	3.39	2.27	3.21	33.09	33.95	24.66	24.34
12-18	0.53	0.21	0.21	0.77	10.36	1.25	1.63	13.22	44.12	11.44	12.86	43.86
18-24	0.12	0.32	0.21	0.39	2.33	4.35	1.28	8.49	14.91	21.22	21.08	45.07
24-36	0.52	0.97	0.67	0.54	15.58	17.2	8.29	19.54	96.73	92.80	76.11	88.71
36-48	0.22	0.22	1.15	0.23	6.77	4.44	8.67	13.91	57.18	49.36	53.78	29.71
48-60	0.25	0.30	0.40	0.30	6.74	9.68	7.97	15.46	100.84	96.76	70.08	87.17
60-72	0.06	0.10	0.12	0.05	3.23	5.03	3.84	3.24	25.33	31.69	31.23	22.30

Table 4. Cumulative excretion (0-t) of Propoxazepam and its metabolites (nmol) in urine during the study period

	Time, h	4	8	12	18	24	36	48	60	72	% of dose
302	Propoxazepam	1.4	2.3	3.2	4.5	4.8	6.1	6.6	7.3	7.4	0.06
	3-hydroxypropoxazepam	15	19	27	55	61	104	122	141	150	1.2
	glucuronide	65	147	237	358	398	663	819	1095	1165	9.5
	total	82	167	267	417	465	773	948	1243	1322	10.8
307	Propoxazepam	1.1	2.6	3.7	4.2	5.0	7.3	7.9	8.6	8.9	0.07
	3-hydroxypropoxazepam	10	16	26	29	41	88	100	127	140	1.1
	glucuronide	46	110	203	234	292	546	681	945	1032	8.4
	total	57	129	232	267	338	641	789	1081	1181	9.6
309	Propoxazepam	1.0	1.7	3.1	3.6	4.2	5.8	8.6	9.6	9.9	0.08
	3-hydroxypropoxazepam	22	24	30	34	38	60	84	106	116	0.9
	glucuronide	17	35	103	138	196	404	551	743	828	6.8
	total	40	61	136	176	237	470	643	858	954	7.8
310	Propoxazepam	1.6	3.0	3.9	5.8	6.7	8.1	8.6	9.4	9.5	0.08
	3-hydroxypropoxazepam	16	40	48	84	108	161	199	241	250	2.0
	glucuronide	23	92	158	278	401	644	725	964	1025	8.4
	Total	40	134	210	368	516	813	933	1215	1284	10.5

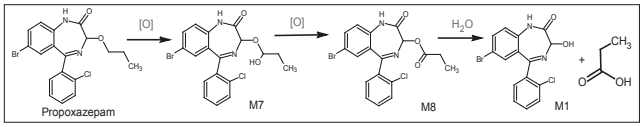
Calculated by the sigma-minus method (linearisation of cumulative excretion data in the coordinates 'ln (Q_{max}-Q_t) from t' with the slope – kel) is 0.04–0.06 h^{-1}. The elimination constant for unchanged propoxazepam is 0.06 h^{-1}, while for 3-hydroxypropoxazepam, both free and conjugated is 0.04 h^{-1}, which provided almost the same rate of their elimination.

DISCUSSION

Propoxazepam is relatively rapidly absorbed after oral administration and the concentration-time profile in serum, despite the high lipophilicity of the compound, can be described by one-compartent model (Fig. 1). C_{max} (~ 22.3 ng/ml) after single 5 mg tablet administration under fasting conditions is reached at 3.5–4 hours, but the long half-life (~30 h) (Tab. 1) suggests its continuous elimination, perhaps due to the lipophilic nature of the parent compound, and is likely to contribute to a long-term therapeutic effect. The high lipophilicity of propoxazepam also determines the significantly high total volume of distribution indicating a significant permeability of the substance to body tissues from the systemic circulation.

Urinary excretion of propoxazepam is characterized by negligible quantities of unchanged compound excreted (0.062% of the administered dose). The pharmacokinetic parameters (in particular, the clearance of unchanged compound from serum and urine) and the maximum amount of urinary excreted propoxazepam, suggest that the parent compound undergoes biotransformation and metabolites are a significant part of the mass balance.

The main metabolite 3-hydroxypropoxazepam is formed during the oxidative dealkylation of the aliphatic moiety of propoxazepam, which is facilitated by the transformation of stable ether group to ester one, which is more capable for hydrolysis, during which the formation of 3-hydroxyderivative takes place [18]:



This process differs from the process of 3C-hydroxylation of 1,4 benzodiazepindes with the methylene group in position '3' of heteroring, which is catalysed with the corresponding monooxygenazes CYP3A4 and CYP2C19 [19]. In this case, the stereoselective formation of 3-hydroxymetabolites takes place due to the asymmetrical carbon in position '3'.

3-Hydroxypropoxazepam was found in dichloromethane extracts of urine as free and, predominantly, glucuronide conjugate, which confirms the assumption of a significant contribution of this pathway to the overall metabolism of propoxazepam. The corresponding glucuronide presence data is consistent with data on high activity and prevalence of key enzymes of this phase in the body.

Since both dealkylation with the formation of 3-hydroxypropoxazepam and subsequent formation of glucuronide are enzymatic processes, the question arises about their connection, because the product of one reaction is the starting substrate for another. In the simplest case, this relationship is expressed by the relationship (correlation) between the concentrations of the substrate and the reaction product.

For rough estimation of the relationship between the transformation processes of propoxazepam with the formation of 3-hydroxypropoxazepam and its glucuronide, correlation coefficients were calculated between changes in their concentrations – the relationship between the concentration of the substrate (propoxazepam) and the formation of 3-hydroxypropoxazepam and further glucuronide (Tab. 5). It is noticeable that the effect of propoxazepam concentration on glucuronide formation is insignificant, but there is a relatively high correlation between propoxazepam concentrations and its first metabolite, 3-hydroxypropoxazepam.

The dynamics of excretion of propoxazepam and its metabolites is almost the same in all studied volunteers and characterized by the presence of several peaks that are similar for 3-hydroxypropxazepam and its glucuronide (Fig. 3). This is probably due to the effect of intestinal-hepatic circulation described for 1,4-benzdiazepine derivatives, in which the compound and its metabolites are excreted with bile into

Table 5. Correlation coefficients of concentrations change of products of metabolic reactions depending on substrate concentration

Volunteer No.	Correlation	3-hydroxy-propoxa-zepam	Glucu-ronide
302	With concentration of propoxazepam	0.54	0.21
	With concentration of 3-hydroxypropoxazepam		0.72
307	With concentration of propoxazepam	0.66	0.41
	With concentration of 3-hydroxypropoxazepam		0.88
309	With concentration of propoxazepam	0.63	0.52
	With concentration of 3-hydroxypropoxazepam		0.69
310	With concentration of propoxazepam	0.27	0.03
	With concentration of 3-hydroxypropoxazepam		0.82

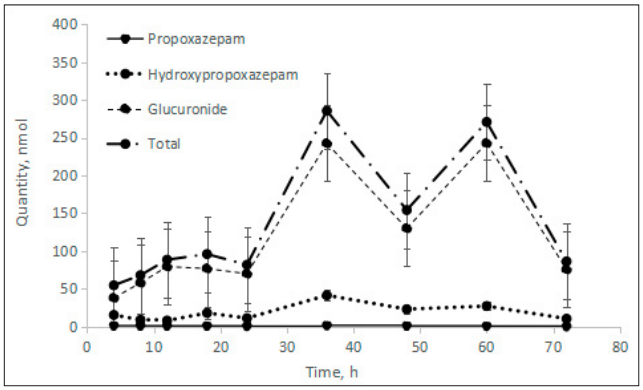


Figure 3. Mean amount (nmol) of urinary excreted propoxazepam, 3-hydroxy-propoxazepam and its glucuronide during the study period (mean ± SD, n=4)

the intestinal lumen, where they undergo partial hydrolysis and subsequent reabsorption.

There are a significant number of glucuronosyltransferase isoforms in the body [20], as the formation of water-soluble and mostly inactive metabolites is the most effective way to remove foreign compounds. In this regard, the formation of glucuronide conjugate of 3-hydroxypropoxazepam is largely determined by the concentration of the starting substrate. In contrast, oxidative dealkylation of propoxazepam occurs due to cytochromes, which, although represented by different isoforms, have relatively low selectivity for xenobiotic metabolism. In addition, the original propoxazepam may form metabolites other than 3-hydroxypropoxazepam, which leads to a lack of relationship between the concentrations of propoxazepam and subsequent metabolites.

On the other hand, the formation of glucuronide conjugate with 3-hydroxypropoxazepam is the predominant process (described processes of oxidation and narrowing of the cycle with the formation of the corresponding quinazolinone refer to the formation of only minor metabolites), therefore the correlation coefficient between changes in concentrations of 3-hydroxypropoxazepam is significantly higher (0.69–0.88) (Tab. 5).

As mentioned earlier, the amount of propoxazepam excreted after 72 hours of exposure was 0.06–0.08% of the administered dose. The amount of free 3-hydroxypropoxazepam excreted is almost an order of magnitude higher – 0.9–2.0%, while the glucuronide-conjugated form is excreted up to 6.8–9.5%. In total, up to 10.5% (7.8–10.8%) of the administered dose of propoxazepam is excreted in the urine as the parent compound and identified metabolites. This ratio of metabolites may indicate a fairly intensive metabolism of propoxazepam in which oxidative dealkylation plays a significant role. This fact must be taken into account when co-administering drugs that cause changes in the activity of enzymes of the first phase of metabolism (cytochrome isoforms).

CONCLUSIONS

Propoxazepam, after a single dose (5 mg) administered orally, showed good tolerability and pharmacokinetically characterized by rapid absorption, slow elimination and low quantities of unchanged parent urinary excreted. The oxidized metabolite (3-hydroxypropoxazepam) and its glucuronide are excreted with urine in total up to 10.5% of the administered dose, which assumes a high degree of metabolism.

Conflict of interest. The authors report they have no conflict of interest.

Author contributions. All the authors contributed to the study design and supervision. Clinical site monitoring was performed by VBL, clinical part performed by IAZ and NPB, obtained data were analysed with the participation of all authors. VBL supervised the metabolites determination. MYG and ASR prepared the manuscript draft. All authors participated in manuscript writing and revision.

Ethical Standards. The study design was approved by the local Ethics Committee of the Clinical Site and the Study Protocol approved by the State Expert Centre of the Ministry of Health of Ukraine (Order N 310 of 23.02.2021).

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