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Elimination kinetics of the novel prodrug cinazepam possessing psychotropic activity in mice

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Abstract:
The kinetics of excretion of the novel tranquilizer cinazepam (3-hydroxy-7-bromo-5-(*ortho*-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one hemisuccinate (I)) in mice after a single administration and different schemes of multiple administration were determined. Mass balance was studied daily in excretions of mice (feces and urine) for 5–10 days. We observed that monoexponential renal excretion of ^{14}C-cinazepam and its metabolites predominated with all dosage regimens. Cinazepam and its metabolites were almost fully (> 90%) eliminated in urine and feces over the period of study (5–10 days), which means that no significant accumulation of the drug in the body occurred. The kinetic parameters of drug excretion were not significantly different after a single injection compared with those following multiple doses of ^{14}C-cinazepam administration. This finding suggests the absence of induction (repression) of enzymatic systems after multiple administration and lack of influence on the kinetic scheme of cinazepam elimination from mice.
In our work, we also presented a modification of the Mansgeldorf's method for analysis of kinetic parameters during multiple administration of the tranquilizer. We demonstrated that our modified approach could be equally and efficiently applied for interpreting experimental data during a single dose administration and after chronic administration of xenobiotics. The use of this method made it possible to evaluate the relative efficiency of elimination processes and to find current values for excretion constants during sampling intervals.

Key words:
cinazepam, elimination, single and multiple administration, mice, modifications of Mansgeldorf's method

Introduction

Cinazepam was synthesized at the Bogatskii Institute of Physical Chemistry of the National Academy of Science of Ukraine. It has now completed the preclinical stage of drug development and started clinical trials [1]. Cinazepam (I) is a novel 1,4-benzodiazepine drug possessing hypnotic and anxiolytic activity without producing myorelaxant side effects. Another important feature of cinazepam is that drug administration does not lead to violation of the sleep structure [2, 11, 20].

Cinazepam has several advantages over conventional benzodiazepines. With cinazepam, the continuity of slow-wave sleep and paradoxical sleep are proportionally increased in contrast to many known hypnosedative drugs, such as diazepam, flunitrazepam

and zopiclone [4, 6–12, 13, 16, 20]. Also, a water-soluble cinazepam salt may be obtained, which is essential for injection preparation.

Previously, we studied the metabolism and pharmacokinetics of cinazepam in experimental animals [23, 28, 29]. However, neither the excretion pattern of cinazepam and its metabolites nor their accumulation in tissues during prolonged administration have been investigated.

In the present study, we investigated the excretion of ^{14}C-cinazepam in mice that received a single injection or one of various multiple-administration regimens of the drug. Conventional approaches, such as the Mansgeldorf's method, Kezdy-Swinbourne method or Guggenheim method (most commonly used in chemical and enzyme kinetics), can be correctly applied for discrimination, qualitative identification and general quantitative analysis of the xenobiotic mass transfer between the body and environment after only a single drug administration [5, 16, 22, 26, 27]. Therefore, to estimate mass transport processes of ^{14}C-cinazepam in the body during multiple administrations for this study, a modification of the conventional methods was proposed.

Materials and Methods

This study was conducted according to the principles of the "Declaration of Helsinki". Experimental protocols were approved by the Ethics Committee of the Pharmacological Committee of Ukraine and carried out in strict accordance with the Ethics Committee regulations for the use of experimental animals.

Animals

The experiments were performed on female outbred mice weighing 18–24 g. The animals were obtained from the breeding facility of the Odessa State Medical University (Odessa, Ukraine) and housed in groups of eight to ten animals per cage. The mice were kept at room temperature under a continuous 12 h light-dark cycle and were provided with food and water *ad libitum*.

Experimental procedures

The pharmacokinetics of cinazepam was studied using labeled ^{14}C-3-hydroxy-7-bromo-5-(*ortho*-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one hemisuccinate (^{14}C-I, 0.30 Ci/mol) (Institute of Physical Chemistry, Odessa, Ukraine).

Cinazepam elimination was investigated after a single injection and during different multiple administration regimens of the drug as follows: group I animals received a single intraperitoneal administration of ^{14}C-I (14 mg/kg) only; group II mice received a single injection of ^{14}C-I (14 mg/kg) after repeated intraperitoneal administrations of non-labeled cinazepam (14 mg/kg per day) for 5 days; and mice from group III received chronic intraperitoneal injections of ^{14}C-I (14 mg/kg per day) for 5 days.

Mass balance was assessed daily in excretions (feces and urine) of mice for 5 (groups I and II) or 10 days (group III). The urine volume and feces weight of each mouse were determined daily. The total radioactivity in fecal and urine samples was measured using a Tri Carb 2700 scintillation photometer (Canberra Packard, USA).

Data analysis

The total radioactivity in excretions after injection of ^{14}C-I (the mean ± SEM) was calculated using Microsoft Excel. The total radioactivity values in the mouse excretions were compared by Student's *t*-test for unpaired data. The results were considered to be statistically significant when $p < 0.05$.

Results

Our pharmacokinetic measurements demonstrate that ^{14}C-I and its radioactive metabolites are almost fully excreted from the body in urine and feces within 5 to 10 days, regardless of the administration schedule (Tab. 1). Renal excretion was found to be a predominant elimination pathway of ^{14}C-I and products of its biotransformation. The fraction of total radioactivity eliminated through the kidneys amounted to 60–75% (Tab. 1). Approximately 33–36% of the total radioactivity was excreted in feces. More than 90% of the injected dose of ^{14}C-product was eliminated within

Tab. 1. Accumulation of the total radioactive material in the excretions of mice in group I, group II (% of doses) and group III (DPM × 10^3)

Time, h	Elimination in urine	Elimination in feces	Total elimination (urine + feces)
		group I	
24	37.79 ± 10.32	18.47 ± 4.82	56.27 ± 11.51
48	53.22 ± 11.03	25.29 ± 5.09	78.52 ± 12.14
72	57.72 ± 11.18	29.45 ± 5.17	87.17 ± 12.31
96	59.05 ± 11.18	32.22 ± 5.28	91.26 ± 12.37
120	59.70 ± 11.20	33.05 ± 5.30	92.75 ± 12.37
		group II	
24	53.98 ± 11.61	18.37 ± 6.80	72.35 ± 13.46
48	70.18 ± 13.05	27.35 ± 6.96	97.52 ± 14.79
72	73.00 ± 13.07	30.47 ± 7.07	103.47 ± 14.86
96	74.38 ± 13.08	34.40 ± 7.14	108.78 ± 14.90
120	75.22 ± 13.08	35.87 ± 7.17	111.09 ± 14.91
		group III	
24	87.30 ± 9.98	159.60 ± 66.95	246.90 ± 67.69
48	244.50 ± 26.96	247.70 ± 17.07	492.30 ± 31.91
72	394.40 ± 36.16	337.10 ± 22.34	731.50 ± 42.51
96	586.10 ± 39.16	446.50 ± 22.01	1032.60 ± 44.92
120	771.20 ± 29.47	541.20 ± 16.51	1312.50 ± 33.78
144	810.70 ± 18.85	587.20 ± 12.62	1398.00 ± 22.68
168	822.00 ± 20.62	616.20 ± 12.22	1438.30 ± 23.99
192	826.40 ± 20.05	629.90 ± 18.32	1456.40 ± 27.16
216	830.30 ± 11.50	633.90 ± 15.50	1464.30 ± 19.30

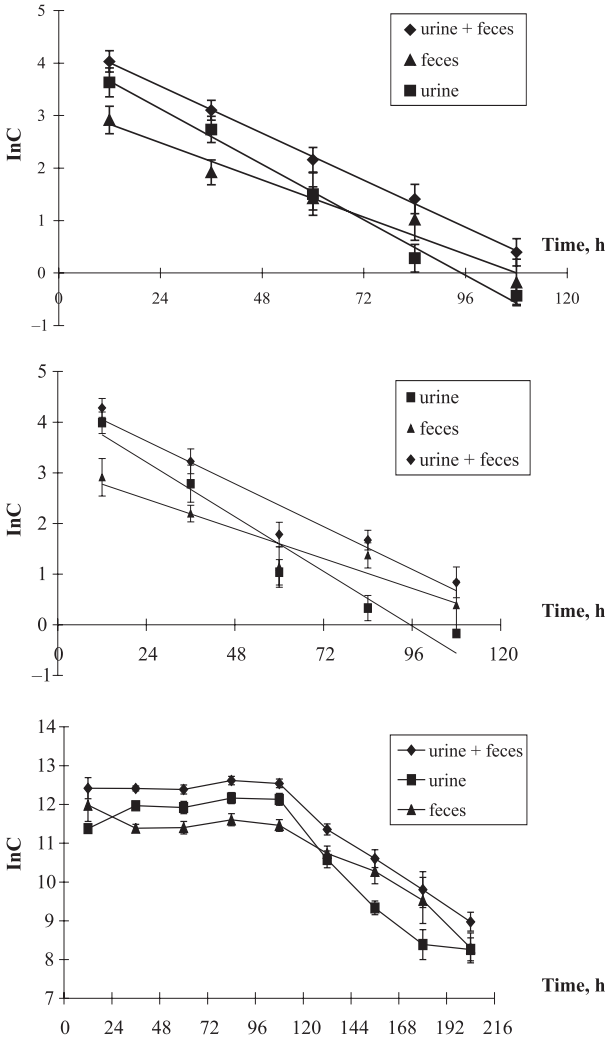

Fig. 1. Elimination kinetics of the total radioactive material (lnC) in the excretions of mice after a single intraperitoneal administration of ^{14}C-cinazepam (group I) or different schemes of multiple administration of ^{14}C-cinazepam or a non-labeled analog for 5 days (groups II and III)

5 days. This observation suggests the absence of a slow-exchange depot compartment for cinazepam.

Approximation of the excretion kinetics in the semi-logarithmic coordinates system (lnC, t) is presented in Figure 1. The results show that the monoexponential decline in the elimination rate is specific and evident for every administration scheme of cinazepam. This decline is typical for the process of elimination *via* urine and feces in animals. Hence, parameters for excretion can be described on the basis of a one-compartment model. The kinetics of the total elimination process (feces and urine) can mostly be defined with the use of parameters specific for the excretion of ^{14}C-products in urine. The observed relations exclude the presence of the peripheral compartment (slow-exchange compartment) in kinetic schemes for the distribution of cinazepam and its metabolites, where reversible accumulation of xenobiotic drugs could take place. A similar kinetic scheme of distribution is characteristic for 3-hydroxy derivatives of 1,4-benzodiazepines, such as lorazepam and oxazepam [8, 14].

The elimination kinetics of total radioactivity are not the same for all routes after administration (Fig. 1). The rate of renal excretion is twice as high as that of fecal elimination for all of the studied schemes (Tab. 2).

The key characteristic of the cinazepam excretion process is its satisfactorily high rate. The biological half-life ($T_{0.5}$) reaches 15–17 h for renal excretion and 23–31 h for fecal elimination (Tab. 2).

Tab. 2. Kinetic parameters of the elimination of the total radioactive material in the excretions of mice after a single or different schemes of multiple administration of ^{14}C-I

Parameters	Elimination in urine	Elimination in feces	Total elimination (urine + feces)
		group I	
$\ln C_0$	4 2 ± 0 .05	3.1 ± 0.06	4.5 ± 0.005
k_e (h^{-1})	0.043 ± 0.0010	0.029 ± 0.0011	0.037 ± 0.0001
$T_{0.5}$ (h)	16.12 ± 2.56	23.91 ± 1.67	18.73 ± 3.06
		group II	
$\ln C_0$	4.1 ± 0.41	3.0 ± 0.12	4.6 ± 0.22
k_e (h^{-1})	0.040 ± 0.004	0.022 ± 0.002	0.036 ± 0.004
$T_{0.5}$ (h)	17.33 ± 3.02	31.51 ± 2.87	19.25 ± 1.78
		group III	
$\ln C_0$	12 4 ± 0.35	11.8 ± 0.03	12.9 ± 0.05
k_e (h^{-1})	0.045 ± 0.006	0.030 ± 0.001	0.038 ± 0.001
$T_{0.5}$ (h)	15.4 ± 1.43	23.10 ± 2.66	18.24 ± 3.02

The quantity of the total eliminated radioactive product for infinite exposition in the first two test groups (single-dose administration of ^{14}C-I), was established on the basis of the modified Mansgeldorf's method. This method and its modification make it possible to evaluate the relative efficiency of the elimination processes and the changes in current excretion constants only in the sample collection intervals after a single dose of a compound has been administered. Methods previously employed for the assessment of the kinetic parameters of excretion of a therapeutic agent, such as the "sigma – minus" method and the "rate – quantity" method, are useless for analyzing the processes that occur after repeated introduction of a drug [22, 25–27]. Hence, developing a method capable of evaluating the changes in parameters during and after the period in which a drug is repeatedly administered offers a chance to obtain valuable information on the preceding processes.

Formal framework of the analysis

The parameters were calculated using Eq. (1) for the measurement intervals of t and $t + \Delta$:

$$\left\{\begin{array}{l} B_{0-t} - B_{0-\infty} = B_{0-\infty} e^{-k_e t} \\ B_{0-t+\Delta} - B_{0-\infty} = B_{0-\infty} e^{-k_e (t+\Delta)} \end{array}\right\} \quad (1)$$

where B_{0-t} and $B_{0-\infty}$ are, respectively, the amounts of substance removed from the organism to the environment by the studied route over the investigation interval and within the interval from time t *to* ∞; k_e is the elimination constant, which characterizes the combined rate of all of the processes responsible for eliminating the preparation from the body; and Δ is the interval of sampling excretions.

If the second equation of the system equation (1) is divided by the first one, then because $e^{-k_e(t+\Delta)} = e^{-k_e t} \cdot e^{-k_e \Delta}$, we have:

$$\frac{B_{0-t+\Delta} - B_{0-\infty}}{B_{0-t} - B_{0-\infty}} = e^{-k_e \Delta} \quad (2)$$

$$B_{0-t} = B_{0-t+\Delta} \cdot e^{k_e \Delta} - \left[B_{0-\infty}\left(e^{k_e \Delta} - 1\right)\right] \quad (2a)$$

$$y_x = b\,x - [a]$$

In the $(y) = B_{0-t}$, $(x) = B_{0-t+\Delta}$ coordinates, the equation (2a) is a linear anamorphosis of the process. The tangent of the angle of inclination (b) of the line corresponds to $e^{k_e \Delta}$; the intersection point of the axis of ordinates, $a = B_{0-\infty}\ (e^{k_e \Delta} - 1)$; the intersection point of the axis of abscissa, $a/b = B_{0-\infty}\ (1 - e^{-k_e \Delta})$; and the intersection point with the bisector line, $[a/(1-b)] = B_{0-\infty}$. The regression analysis made it possible to calculate the values for $B_{0-\infty}$ and k_e as well as the relative efficiency of the elimination of the compound and its metabolites in urine:

$$\omega_1 = \frac{B_{(0-\infty)1}}{B_{(0-\infty)1} + B_{(0-\infty)2}} \quad (3)$$

and in feces:

$$\omega_2 = \frac{B_{(0-\infty)2}}{B_{(0-\infty)1} + B_{(0-\infty)2}} \quad (4)$$

for the first two groups of experimental animals (Tab. 3).

As can be seen from these calculations, the compound and its metabolites are almost completely eliminated from the mouse through the studied routes (Tab. 3).

These parameters show a significantly higher rate and relative efficiency of renal excretion of the compound and its metabolites compared with the process of elimination *via* feces. The non-radioactive compound, when introduced during the previous 5 days, had no effect on the kinetic parameters of the elimination processes of ^{14}C-I administered in a single dose ($p \leq 0.001$).

Tab. 3. Results of the regression analysis of the elimination kinetics of the total radioactive material in the excretions of mice after a single or different schemes of multiple administration of ^{14}C-I (14 mg/kg) (A) or a single injection of ^{14}C-I (14 mg/kg) in mice that received intraperitoneal injections of non-labeled cinazepam (14 mg/kg a day) during the previous 5 days (B)

Excretes	A			B		
	$B_{0-\infty}$, (% doses)	ω	k, h^{-1}	$B_{0-\infty}$, (% doses)	ω	k, h^{-1}
Urine	60.5 ± 3.98	0.63 ± 0.06	0.041 ± 0.01	75.2 ± 6.43	0.65 ± 0.1	0.053 ± 0.01
Feces	34.2 ± 2.23	0.36 ± 0.031	0.032 ± 0.004	36.8 ± 4.56	0.32 ± 0.048	0.028 ± 0.004
Urine + feces	93.2 ± 4.43	–	0.038 ± 0.01	110.5 ± 7.56	–	0.044 ± 0.01

Taking the logarithms of Eq. (2), the change in the values of k_e in the intervals of sampling excreta can be determined after a single dose of labeled compounds (groups I and II):

$$k_{e\Delta} = -\frac{1}{\Delta}\ln\left[\frac{B_{0-\infty} - B_{0-t+\Delta}}{B_{0-\infty} - B_{0-t}}\right] \quad (5)$$

where $k_{e\Delta}$ is the elimination constant, measured in the time interval (Δ).

Based on the determination of this parameter, one can estimate the change in kinetic processes under single-dose administration conditions and under prolonged administration of drugs (e.g., the acceleration of elimination as a result of inducing enzymatic systems).

Tab. 4. Current constants of the elimination of ^{14}C-cinazepam and its metabolites from mice after a single administration of ^{14}C-I (group I) or a single injection of ^{14}C-I (14 mg/kg) in mice that received intraperitoneal injections of non-labeled cinazepam (14 mg/kg per day) during the previous 5 days (group II)

Time, h	Urine	Feces	Urine + feces
		group I	
12	0.041 ± 0.008	0.032 ± 0.002	0.038 ± 0.004
36	0.047 ± 0.0075	0.024 ± 0.003	0.038 ± 0.003
60	0.040 ± 0.007	0.026 ± 0.002	0.037 ± 0.0025
84	0.027 ± 0.004	0.036 ± 0.0015	0.047 ± 0.006
108	0.025 ± 0.005	0.023 ± 0.003	0.063 ± 0.01
		group II	
12	0.051 ± 0.009	0.028 ± 0.002	0.044 ± 0.009
36	0.056 ± 0.006	0.027 ± 0.004	0.045 ± 0.004
60	0.029 ± 0.004	0.016 ± 0.001	0.025 ± 0.002
84	0.029 ± 0.003	0.040 ± 0.007	0.058 ± 0.005
108	0.040 ± 0.0065	0.038 ± 0.007	0.057 ± 0.008

As can be seen from the analysis of the experimental data (Tab. 4), there was no systematic change in the value of the rate constant of ^{14}C-product elimination from the mouse after a single dose of ^{14}C-cinazepam (A) or against the background created by administrating its non-radioactive analogue (B). This finding indicates that the prior daily administration of non-labeled cinazepam has no effect on this parameter.

The formal framework can be simplified if the kinetics of excretion of that drug from experimental animals after its multiple administrations is studied in compliance with the following conditions:

a) the time intervals for collecting excreta should be equal to each other (and set as some value Δ) and equal to time intervals for successive doses of the xenobiotic drug administered, i.e., samples should be collected directly before introducing another dose of the studied substance; and

b) the administered doses ($Q_1 = Q_2 = ... Q_i = Q$) should be equal to each other.

The amount of the substance that will be eliminated into the environment by the route under investigation (e.g., *via* urine) for an infinitely long exposition ($B_{0-\infty}$) is equal to:

$$B_{0-\infty} = \omega\sum_{i=1}^{i=n} Q \quad (6)$$

where ω is relative efficiency of a given route of excretion; and $\sum_{i=1}^{i=n} Q$ is the sum of (n) doses (Q) of the substance introduced to the organism [in our case, in equal time intervals (Δ)].

If the kinetic scheme is linear and $\sum_{i=1}^{i=n} Q = nQ$, then the amount of the substance that is eliminated in urine

over the time interval from t to $t + \Delta$ should be determined from the following equation:

$$(B_{0-t+\Delta} - B_{0-t}) = (i\omega Q - B_{0-t})(1-e^{-k_e\Delta}) \quad (7)$$

where i is an amount of successively introduced doses in the experimental interval from 0 to $t\left(i=\frac{t}{\Delta}+1\right)$, and k is the total constant for elimination of the xenobiotic from the organism through a given route.

The first dose was introduced at $t = 0$ ($I = 1$), the second at $t = \Delta$ ($I = 2$), the third at $t = 2\Delta$ ($I = 3$), etc.

From Eq. (7), we obtain the resulting linear equation:

$$\frac{B_{0-t+\Delta}}{i} = \frac{B_{0-t}}{i}\left(e^{-k_e\Delta}\right) + \left[\omega Q\left(1-e^{-k_e\Delta}\right)\right] \quad (8)$$
$$y_{(x)} = x(b) + [a]$$

The tangent of the angle of inclination of the regression line is $\left(e^{-k_e\Delta}\right)$, intersecting the y- and x-axes at points $y_0 = \omega Q\left(1-e^{-k_e\Delta}\right)$ and $x_0 = -\omega Q\left(\frac{1-e^{-k_e\Delta}}{e^{-k_e\Delta}}\right)$ respectively.

For $t \to \infty$; $\frac{B_{0-t}}{i} \to \frac{B_{0-t+\Delta}}{i}$, the regression line will cross a line segment bisector of the quadrate at the point with coordinates $y_{(x)} = x_{(y)} = \omega Q$ (Eq. 8). The diagram of this method is identical and formally compatible with the Mansgeldorf's method, which is used to interpret experimental data for the elimination kinetics of a xenobiotic introduced in single doses ($i = 1$) [5, 29].

Regression analysis of the experimental data (Fig. 2) showed that the elimination rate of the total radioactivity in feces decreased non-linearly over the time interval under prolonged administration of the substances (the first five experimental points of the curves).

The relative efficiency of the excretion processes *via* feces and urine during an infinitely long-term administration of ^{14}C-I is approximately equal to ~1:1.5. When the administration of the tested substance is stopped, the linearity of the kinetic system is restored (Fig. 2).

The excretion processes of ^{14}C-I and its metabolites in the urine are characterized by a linear kinetic scheme following the introduction of multiple doses of a substance as well as after the termination of its administration.

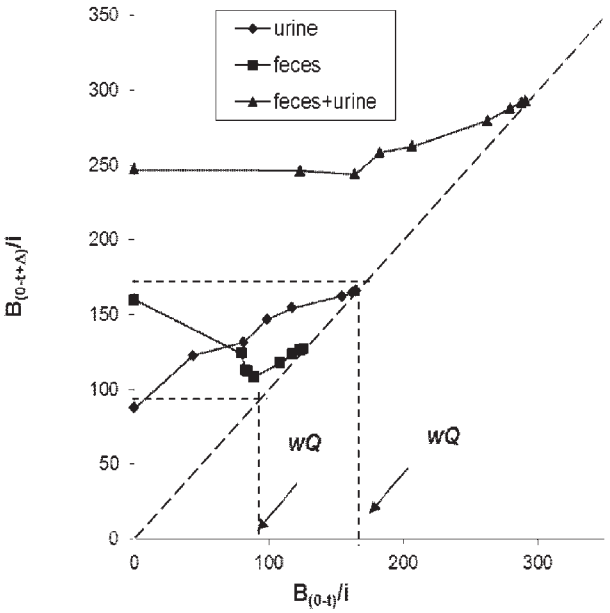

Fig. 2. The elimination kinetics of the total radioactive material in the excretions of mice after a multiple-administration regimen of cinazepam by a regression method (modification of Mangelsdorf's method)

Discussion

The aim of this work was to study the excretion kinetics of the new tranquilizer cinazepam in mice after single or multiple administrations of the drug. The investigation of the pharmacodynamics of cinazepam demonstrated a high anxiolytic, sleep-inducing effect and low acute toxicity [2, 11, 15, 19, 20]. However, the results of radioligand binding to $GABA_A$ receptor subunits showed that the EC_{50} of cinazepam was one order of magnitude lower than the values for the well-known sleep-inducing drugs, like nitrazepam and phenazepam. [3, 4, 18].

The results of studies on the biotransformation of a compound in the body of experimental animals can explain the differences between the parameters of the compound's pharmacological effect and the radioligand analysis.

Previously, we have shown that in mice and rats, the predominant metabolite is 7-bromo-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-3H-1,4-benzdiazepine-2-one (II) and the products of its oxidation (Fig. 3).

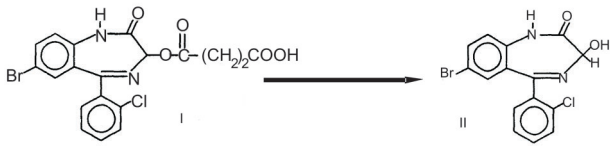

Fig. 3. Structures of the parent compound (I) and its metabolite (II)

The metabolite II shows high activity and high affinity for the benzodiazepine receptor. The rate of the formation process of the 3-hydroxy metabolite and its efficiency are sufficiently high. Only some 5% of the basic compound can be detected in the blood of mice as early as after 30 min of the experiment. These studies allows us to consider cinazepam as a prodrug with an unlimited rate of conversion to the active metabolite. [23, 29].

In this study, we used a radioactive analog containing an isotope of [^{14}C] in position 2 of the heterocyclic ring, which would not be subjected to elimination from the compound in the process of its biotransformation.

The use of radioactive label in this position made it possible to use the total radioactivity to analyze the elimination kinetics for all of the metabolites formed. Three schemes offered the chance to trace the "life route" of the single dose (schemes of administration 1), to evaluate the induction or inhibition effects of enzymes that metabolize the compound based on the parameters of the excretion kinetic scheme (schemes of administration 2) and to evaluate the effects of accumulated doses for the repeated administration model (schemes of administration 3).

The characteristic of excretion processes of cinazepam was a predominance of renal excretion (60%) over the elimination with feces (33%) (Tab. 1). An analogous process is specific for 3-hydroxybenzodiazepines, such as oxazepam and lorazepam, which undergo glucuronide conjugation. These results demonstrate that cinazepam has a median elimination half-life of 16–23 h [8, 14, 17].

The elimination kinetics of the total radioactivity in urine and feces are characterized by a linear scheme for all methods of administration. The elimination processes through given routes are not parallel. Preliminary introduction of a non-radioactive analogue does not affect the kinetics of excretion or induction or inhibit the enzyme systems.

The modified Mansgeldorf's method can be efficiently and successfully employed in the interpretation of the experimental data obtained both for single and multiple doses introduced and for after the chronic administration of a xenobiotic has stopped. The use of that method made it possible to evaluate the relative efficiency of the elimination processes and to find the current values for excretion constants over the sampling intervals.

In the case of a single dose of cinazepam, no reliable changes were noted in the values of the excretion rate constants for sampling intervals and for both routes of elimination. After repeated administration of cinazepam, the rate of its elimination process in feces declined in a non-linear way. Linear kinetic scheme of 14-C-I excretion in feces is restored after the multiple administrations of the drug was stopped (Fig. 2). The linear scheme of the kinetics is also characteristic of urine elimination. Thus, the use of the proposed method showed that with daily administration of the prodrug, a decrease in the relative efficiency of excretion of ^{14}C-products in the feces of animals is observed. This particularity of the excretion process was not revealed earlier for the derivatives of 1,4-benzodiazepine.

References:

1. Andronati SA, Yakubovskaya LN, Yavorsky AS, Pavlovski VI, Golovenko MYa, Seredenin SB, Voronina TA et al.: Hemisuccinate – 3-hydroxy-7-bromo-5 – (ortho-chloro)-phenyl-1.2-dihydro-3H -1,4-benzdiazepin-2-one, which has a hypnotic, sedative, tranquilizing activity. Patent 19803, Ukraine MKI 5CO7 D 243/14, A 61 K31/55. od 25.12.1997. Bull. 6, 18s.
2. Andronati SA, Karasyova TL, Popova KI, Makan SYu, Boyko IA: GABA-ergic hypnotics (Russian). Bulletin of Psychiatry and Psychopharmacotherapy, 2004, 1, 6–17.
3. Blednov IuA, Kosach IV, Seredenin SB: The effect of 1,4-benzodiazepine derivatives on ^{35}S-tetrabutylbicyclophosphorothionate binding in the brain of inbred mice with differing emotional stress reactions (Russian). Eksp Klin Farmakol, 1997, 60, 3–6.
4. Boyko IA, Makan SYu, Smulsky SP, Andronati SA: The study of affinity for the benzodiazepine sites of GABA A receptors of the CNS and functional activity of potential hypnotic – cinazepam (Russian). Bull of the Odessa Nat Univ, 2005, 10.49–58.
5. Cornish-Bowden A: Fundamentals of Enzyme Kinetics, Portland Press Ltd., London, 2004.

6. Danneberg P, Weber KH: Chemical structure and biological activity of the diazepines. Br J Clin Pharmacol, 1983, 16, Suppl 2, 244.
7. Davies M, Newell JG, Derry JMC, Martin IL, Dunn SMJ: Characterization of the interaction of zopiclone with γ-aminobutyric acid type-A receptors. Mol Pharmacol, 2000, 58, 756–762.
8. Dingemanse J, Voskuyl RA, Langemeijer MW, Postel-Westra I, Breimer DD, Meinardi H, Danhof M: Pharmacokinetic-pharmacodynamic modelling of the anticonvulsant effect of oxazepam in individual rats. Br J Pharmacol, 1990, 99, 53–58.
9. Dujardin K, Guieu JD, Leconte-Lambert C, Leconte P, Borderies P, de La Giclais B:. Comparison of the effects of zolpidem and flunitrazepam on sleep structure and daytime cognitive functions. A study of untreated unsomniacs. Pharmacopsychiatry, 1998, 31, 14–18.
10. Ferentinos P, Paparrigopoulo T: Zopiclone and sleepwalking. Int J Neuropsychopharmacol, 2009, 12, 141–142.
11. Godlevsky LS, Karasyova TL, Popova KI, Andronati SA: Influence of cinazepam on sleep-wake up cycle in rats (Russian). Progress of Biology and Medicine, 2005, 2, 22–26.
12. Gottesmann C: Detection of seven sleep-waking stages in the rat. Neurosci Biobehav Rev, 1992, 16, 31–38.
13. Gottesmann C, Gandolfo G, Arnaud C, Gauthier P: The intermediate stage and paradoxical sleep in the rat: influence of three generations of hypnotics. Eur J Neurosci, 1998, 10, 409–414.
14. Guentert TW: Time-dependence in benzodiazepine pharmacokinetics. Mechanisms and clinical significance. Clin Pharmacokinet, 1984, 9, 203–210.
15. Karaseva TL, Popova LV, Onufrienko OV, Andronati KS, Andronati SA: Experimental study of the development of tolerance to cinazepam (Russian). Reports of NAN of Sciences of Ukraine, 2008, 7, 157–161.
16. Keleti T: Basic enzyme kinetics, Akademiai Kiado, Budapest, 1986.
17. Lasoń W, Dudra-Jastrzębska M, Rejdak S, Czuczwar J: Basic mechanisms of antiepileptic drugs and their pharmacokinetic/pharmacodynamic interactions: an update**.** Pharmacol Rep, 2011, 63, 271–292.
18. Makan SY, Boiko I, Smulskii SP, Andronati CA: Effect of cinazepam administration on the ligand affinity of neuromediator system receptors in rat brain. Pharm Chem J, 2007, 41, 249–252.
19. Maykova AV, Onofriychuk AA, Sulakova OB, Pavlichenko OD: Determination of anesthetic cinazepam properties in the rat (Russian). Bull Odessa Nat Univ, 2006, 11, 228–234.
20. Maykova AV, Zhuk OV: Anticonvulsant activity of cinazepam on the model seizures with different mechanism of forming (Russian). Bull Odessa Nat Univ, 2005, 10, 165–175.
21. Nachkebia N, Dzadzamia Sh, Chkhartishvili E, Mchedlidze O, Oniani T: Influence of diazepam on different behavioral states of sleep-waking cycle. Georgian Med News, 2009, 4, 94–99.
22. Onishi S, Kawade N, Itoh S, Isobe K, Sugiyama S, Hashimoto T, Narita H: Kinetics of biliary excretion of the main two bilirubin photoproducts after injection into Gunn rats. Biochem J, 1981, 198, 107–112.
23. Schukin SI, Zinkovsky VG: Biotransformation and biokinetics of the cinazepam – novel drugs possessing psychotropic activity. Abstracts of Joint Meeting on Medicinal Chemistry. Kraków, 2003, 60.
24. Sivertsen B, Omvik S, Pallesen S, Nordhus IH, Bjorvatn B: Sleep and sleep disorders in chronic users of zopiclone and drug-free insomniacs. J Clin Sleep Med, 2009, 5, 349–354.
25. Tarka SM Jr, Arnaud MJ, Dvorchik BH, Vesell ES: Theobromine kinetics and metabolic disposition. Clin Pharmacol Ther, 1983, 34, 546–555.
26. Varfolomeev SD, Gurevich KG: Biokinetics: Practical course, Fair-press, Moscow, 1999.
27. Welling PG: Graphic methods in pharmacokinetics: the basics. J Clin Pharmacol. 1986, 26, 510–514.
28. Zhuk OV, Zinkovsky VG, Schukin SI: Kinetic analysis of the process elimination after single and different schemes of multiple administration of cinazepam – a novel prodrug possessing psychotropic activity. Eur Neuropsychopharmacol, 2005, 15, Suppl 2, 155–156.
29. Zhuk OV, Zinkovsky VG, Schukin SI, Sivachenko AV: Biotransformation, pharmacokinetics and pharmacodynamics of cinazepam. Pharmacol Rep, 2007, 59, Suppl 1, 60–61.
30. Zin'kovskii VG, Vasilinin GB, Stankevich EA, Golovenko NYa, Zhuk OV: Pharmacokinetics of bromonordiazepam and its ^{14}C– analog in single and prolonged patterns of administration. Pharmaceut Chem J, 1988, 22, 197–204.

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