# COMPARATIVE ANALYSIS OF THE PHARMACOKINETICS OF UNSUBSTITUTED $N^1$-DERIVATIVES OF 1,4-BENZODIAZEPINE

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The pharmacokinetics of 1,4-benzodiazepine derivatives has now been adequately studied. However, no data generalizing these results and indicating similarity or difference between the metabolic pathways or kinetics of distribution of preparations of the 1,4-benzodiazepine series are yet available. There are several reasons for this. First, these preparations contain different substituents in their aromatic nuclei and their heterocyclic ring. Second, investigations have been conducted on many species of experimental animals, and different methods of mathematical analysis have accordingly been used.

The aim of this investigation was to make a general assessment of the pharmacokinetics (dependent on structure of the model) of some 1,4-benzodiazepine derivatives. For this purpose the range of substances studied was limited to $N^1$-unsubstituted derivatives. Most attention was paid to the pharmacokinetics of 7-bromo-5-phenyl-1-H-1,3-dihydro-1,4-benzodiazepin-2-one (I), which is the principal metabolite of the peptidaminobenzophenones [4] and a convenient structural object with which to compare the pharmacokinetics of phenazepam, nordiazepam, bromazepam, etc.

## EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice weighing 18-20 g. $^{14}$C-I (0.78 Ci/mole) was injected intraperitoneally in Tween emulsion in a dose of 1.4 mg/kg body weight. The animals were decapitated 10, 20, 30, 60, 120, 240, and 360 min later. Concentrations of total, water-soluble, and protein-bound samples of radioactivity and total concentrations of free metabolites in blood plasma and brain homogenates (1:5) of the animals were determined by methods described previously [2] on the LS-100C instrument (Beckman, USA). The content of I and its metabolites was determined by thin-layer zonal radiochromatography on Silufol UF-254 plates after preliminary (to ensure complete extraction of the compounds to be studied, namely 0.95) successive extraction of the plasma and homogenates with chloroform. Chromatography was carried out in a system of chloroform—acetone—aqueous ammonia (70:30:0.1). The techniques and metrologic characteristics of the methods were described previously [2, 6]. The data were analyzed in accordance with algorithms in [7, 10].

## EXPERIMENTAL RESULTS

Radiochromatographic analysis of chloroform extracts of blood plasma samples and brain extracts of mice receiving an injection of $^{14}$C-I showed the presence of the original compound ($R_f$ = 0.75), its 3-hydroxy metabolite (II) with $R_f$ = 0.45, and the sum of the aromatic hydroxylation product (III), compounds with $R_f$ values of 0.00-0.15.

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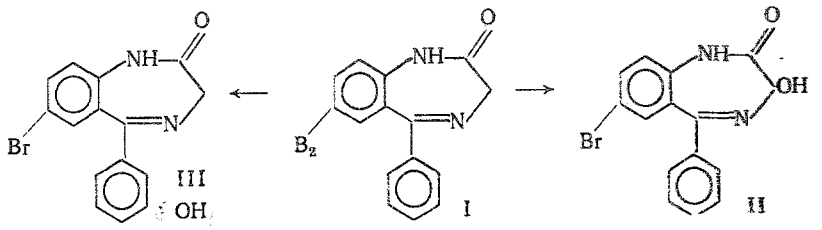
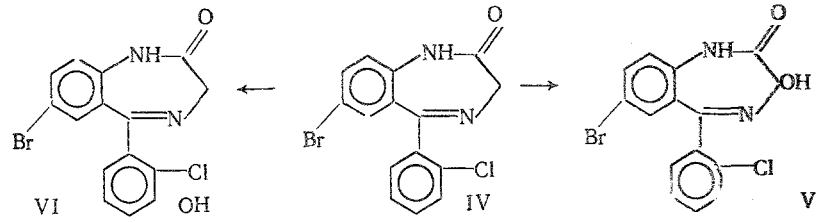


Metabolites I and II were found in the form of conjugated glucuronate and sulfate, whereas III also was bound with proteins of mouse blood plasma and brain.



Similar results were obtained by radiochromatographic analysis of phenazepam (IV) and its corresponding metabolites (V and VI) in mouse organs and tissues [5, 8, 9]. Besides the original substance, its 3-hydroxy metabolite (VIII) also was found in the plasma and brain of mice after administration of nordiazepam (VII) [9]. Analysis of metabolites of bromazepam (IX) and its 3-hydroxy derivative (X) is described in [12]. In all the above-mentioned investigations significant amounts of the corresponding quinazolin-2-ones were not found, although the process of further oxidation of the heterocyclic ring of the 3-hydroxy metabolites of 1,4-benzodiazepines in mice does take place [9, 11]. Consequently, pathways of biotransformation of the majority of $N^1$-unsubstituted derivatives of 1,4-benzodiazepine are similar.

The change in the level of total radioactive material, and also of I and its metabolites (II and III) observed in the mice in the course of the experiment (10-360 min) can be divided conventionally into two phases: the phase of rapid change of concentration of $^{14}C$-derivatives (10-120 min) and the phase of a stationary concentration (120-360 min) in the brain and blood plasma (Table 1; Fig. 1). Pharmacokinetic curves of the compounds studied differed significantly and can also be divided into two groups. The content of total radioactivity and the sum of free metabolites and of I are characterized by rising to a peak concentration after 20 min, followed (20-120 min) by a rapid fall. For concentrations of II, III, and the sum of water-soluble and protein-bound $^{14}C$-metabolites a rapid rise was observed between 10 and 120 min, followed by maintenance of a steady level. Curves of a similar character were observed in an investigation [5] of the pharmacokinetics of IV, V, and VI, and of free, water-soluble, and protein-bound metabolites IV, and an enzyme-kinetic model has been described. Analysis of the kinetics of distribution of IX and X was based on linear kinetic schemes [12] in an investigation described in [11]. The shape of the distribution processes of VII and VIII observed was similar, but they were not subjected to pharmacokinetic analysis. The main differences were a slower course of the first phase of distribution (180 and 360 min) of VII and IV compared with I and a more rapid course (60 min) for IX and X. A characteristic feature of the pharmacokinetics of I was the stationary ratio observed between concentrations of total radioactivity, and the sum of free, water-soluble and protein-bound metabolites I, II, and III. The values of the distribution constants (brain/blood plasma), which differed for the original compounds and their metabolites, were invariant relative to the time of the experiment or the concentrations of the compounds indicated. This suggests a high rate of metabolism and excretion compared with intake processes, and the reversibility of the processes observed.

A similar rule was found in previous studies of the pharmacokinetics of IV in mice of different lines and hybrids [5, 8, 9]. These parameters are independent of the blast factor, and the values of the distribution constants of IV, V, and VI are lower than those of I, II, and III, being 1.88, 1.56, and 1.04, respectively. A similar dependence also was observed in [12], when values of the distribution constants (brain/blood plasma) for IX and X were found to be 0.88-1.2 and 0.57-1.2.

For aromatic hydroxylation products of I and IV the values of the distribution constants were lower than those of the original compounds and their 3-hydroxy metabolites [5, 9] (Fig. 1).

TABLE 1. Concentrations (in cpm × $10^3$•g/ml) of Total Radioactivity (A), Sums of Free (B), Water-Soluble (C), and Protein-Bound Biological Substrates (D) of Metabolites I in Mouse Brain (1) and Blood Plasma (2), and also Ratio of these Values (3) in the Course of the Experiment after Intraperitoneal Injection of I (0.78 Ci/mole) in a Dose of 1.4 mg/kg (M ± m, n = 6)

| Time of experiment, min | A | | | B | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| 10 | 2,36±0,12 | 7,89±0,62 | 3,34 | 2,42±0,13 | 5,24±0,34 | 2,17 |
| 20 | 3,56±0,35 | 12,11±1,66 | 3,40 | 3,68±0,12 | 9,31±1,16 | 2,53 |
| 30 | 3,28±0,34 | 9,68±1,35 | 2,95 | 2,53±0,31 | 6,88±0,79 | 2,72 |
| 60 | 2,41±0,25 | 8,05±0,51 | 3,34 | 2,52±0,24 | 5,80±0,19 | 2,30 |
| 120 | 1,76±0,11 | 6,00±0,28 | 3,41 | 1,35±0,13 | 4,39±0,22 | 3,25 |
| 240 | 1,90±0,11 | 6,10±0,98 | 3,21 | 1,33±0,23 | 4,30±0,17 | 3,23 |
| 360 | 2,18±0,16 | 5,95±0,42 | 2,73 | 1,75±0,21 | 3,96±0,26 | 2,26 |

| Time of experiment, min | C | | | D | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| 10 | 0,51±0,13 | 1,01±0,11 | 1,98 | 0,20±0,05 | 1,91±0,54 | 9,55 |
| 20 | 0,55±0,17 | 1,55±0,26 | 2,82 | 0,19±0,03 | 1,94±0,24 | 10,21 |
| 30 | 0,58±0,12 | 1,46±0,24 | 2,52 | 0,13±0,03 | 1,68±0,39 | 12,92 |
| 60 | 0,50±0,10 | 1,09±0,12 | 2,18 | 0,47±0,12 | 1,21±0,13 | 2,57 |
| 120 | 0,24±0,06 | 0,86±0,12 | 3,58 | 0,17±0,05 | 1,13±0,09 | 6,65 |
| 240 | 0,31±0,07 | 0,88±0,12 | 2,84 | 0,32±0,08 | 1,22±0,16 | 3,81 |
| 360 | 0,23±0,06 | 0,65±0,10 | 2,83 | 0,31±0,06 | 1,09±0,10 | 3,52 |

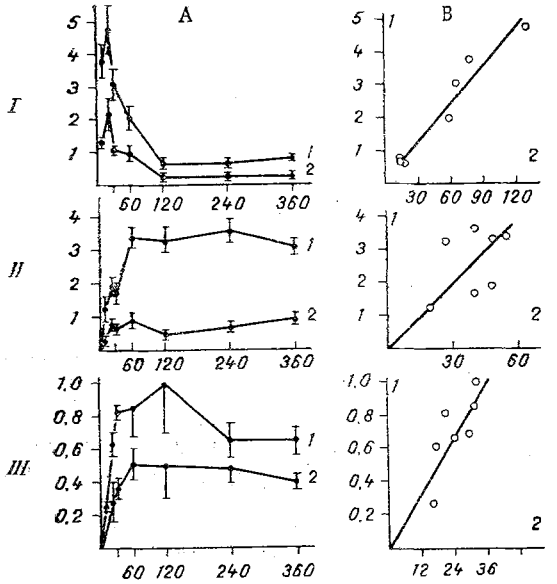



Fig. 1. Kinetics (A) and relative concentration (in cpm × $10^3$•g/ml) of original compound (I), its 3-hydroxy metabolite (II), and sum of aromatic hydroxylation products of I (III) in mouse brain (1) and blood plasma (2) (each point represents M ± m, n = 6) after intraperitoneal injection of $^{14}$C-I (0.78 Ci/mole) in a dose of 1.4 mg/kg. Abscissa, time of experiment (in min).

No significant correlation was found between values of these constants for compounds I, II, IV, V, VII, and VIII and calculated and experimental values of the logarithms of their distribution in an octanol—water system [11], or in their pharmacological activity and affinity for benzodiazepine receptors [12]. Another characteristic parameter is the linear relationship between injected dose and concentration of IV and IX in the mouse brain and blood plasma [3, 12]. The coefficient of regression was found to be 1.2 and 0.84, respectively.

The shape of the dependence remained the same when different forms of the preparation were administered by different routes.

The values given in [4] are not interconnected with values of distribution constants for brain/blood plasma or with dependence of concentration in these media on injected doses. Consequently, these parameters (characterizing the pharmacokinetic phase of interaction between drug and organism), together with data taking account of the intensity of metabolism and also with the results of pharmacological [1, 2, 4] and radioreceptor [3, 4] experiments, provide independent parameters for pharmacological prediction.

## LITERATURE CITED


1. S. A. Andronati, V. M. Chepelev, L.N. Yakubovskaya, et al., Bioorg. Khim., No. 10, 1357 (1983).
2. N. Ya. Golovenko and V. G.Zin'kovskii, in: Phenazepam, Kiev (1982), pp. 32-86.
3. N. Ya. Golovenko, V. G. Zin'kovskii, O. V. Zhuk, and V. A. Sozinov, in: Pharmacology of Gamma-Aminobutyric Acid Derivatives [in Russian], Tartu (1983), pp. 22-24.
4. N. Ya. Golovenko, O. P. Rudenko, M. Yu. Totrova, et al., in: Protein and Peptide Chemistry [in Russian], Riga (1983), p. 301.
5. N. Ya. Golovenko, V. G. Zin'kovskii, and S. B. Seredenin, Khim.-Farm. Zh., No. 12, 14 (1980).
6. O. V. Zhuk, V. G. Zin'kovskii, and N. Ya. Golovenko, Khim.-Farm. Zh., No. 3, 361 (1983).
7. N. A. Plokhinskii, Algorithms of Biometrics [in Russian], Moscow (1980).
8. S. B. Seredenin, V. G. Zin'kovskii, V. A. Batyttov, et al., Byull. Éksp. Biol. Med., No. 10, 450 (1981).
9. S. B. Serdenin, V. G. Zin'kovskii, N. Ya. Golovenko, and I. V. Rybina, Khim.-Farm. Zh., No. 9, 23 (1981).
10. V. N. Solov'ev, A. A. Firsov, and V. A. Filov, Pharmacokinetics [in Russian], Moscow (1980).
11. F. Marcucci, E. Mussini, R. Fanelli, and S. Garatini, Biochem. Pharmacol., 19, 1847 (1970).
12. M. A. Schwartz, W. R. Pool, D. L. Hane, and A. E. Postma, Drug. Metab. Dispos., No. 1, 31 (1974).