Biokinetics of Transdermal 3-Hydroxyphenazepam

N. Ya. Golovenko, V. B. Larionov, I. A. Kravchenko, N. V. Ovcharenko, and A. I. Aleksandrova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 9, pp. 295-298, September, 2002
Original article submitted January 24, 2002

The kinetics of labeled 3-hydroxyphenazepam (active phenazepam metabolite) after its intravenous and transdermal administration was studied. Biphasic kinetic of drug distribution in the organism was revealed; pharmacokinetic parameters and biological availability (1.32± 0.12 for the plasma and 1.21±0.10 for the brain) were determined on the basis of total radioactivity of the plasma and brain.

Key Words: *transdermal administration; 3-hydroxyphenazepam; bioavailability*

Transdermal administration of drugs producing systemic effects attracts much recent attention [5-7]. Delivery of drugs through the skin is carried out by means of transdermal therapeutic systems (TTS).

Transdermal administration is characterized by numerous advantages: prolonged effect of the drug; maintenance of its therapeutic concentration; atraumatic administration and the possibility of its discontinuation by removal of the TTS from the skin; slow drug metabolism during its first passage through the liver; the possibility of using highly active drugs irritating the gastrointestinal tract and characterized by short half-life period.

Agents used in TTS have to meet certain requirements: easy penetration through the skin and high efficiency at low concentrations [4]. Phenazepam meets these requirements [1]. We previously investigated the possibility of using this preparation with TTS [2,3]. 3-Hydroxyphenazepam (3-HOP), a product of biotransformation of phenazepam possesses tranquilizing and anticonvulsive activities. Hence, this compound can be used as the active component of TTS.

We investigated the distribution of labeled 3-HOP in the plasma and brain in mice after its intravenous and transdermal administration and evaluated pharmacokinetic parameters and absolute bioavailability of its transdermal form.

I. I. Metchnikov National University; A. V. Bogatskii Physico-Chemical Institute, National Academy of Sciences of Ukraine, Odessa

MATERIALS AND METHODS

TTS used in our study was a hydrogel matrix consisting of polyvinylalcohol and propylene glycol (plasticizer) and containing a certain concentrations of ^{14}C-3-HOP. 3-HOP solution in propylene glycol was mixed with aqueous solution of polyvinyl alcohol and the matrices were made using pouring-drying technique.

For pharmacokinetic studies, ^{14}C-3-HOP (specific activity 0.2 Ci/mol, radiochromatographic purity 75.2±6.4%) was added to the TTS.

The experiments were carried out on mice. TTS was applied on the back between the scapulae. The skin was previously shaven. The animals were kept under conditions with free access to water and food and excluding grooming.

The applied dose of 3-HOP was 16 mg/kg (0.4 mg/cm^2). The concentrations of radioactive products in the plasma and brain were evaluated 0.25, 0.5, 1, 3, 6, 8, 12, 24, and 48 h after TTS application by liquid scintillation photometry on a TRI-CARB 2700 TR device (Canberra Packard).

Diffusion of 3-HOP into the body was evaluated by direct measurement of total radioactivity. To this end brain sample was hydrolyzed in 1 ml 98% formic acid on boiling water bath for 1 h; 0.5 ml hydrolysate was transferred into scintillation vials and 1 ml toluene-triton X-100 mixture (1:1) and 10 ml toluene-ethanol scintillator were added. For evaluation of plasma 3-HOP concentration, the blood was collected into

0007-4888/02/1343-0254$27.00

heparin-rinsed centrifuge tubes, centrifuged for 10 min at 8000 rpm, and 0.5 ml plasma was transferred into scintillation vials with 1 ml toluene-triton X-100 mixture (1:1) and 10 ml toluene-ethanol scintillator. The distribution of ^{14}C-3-hydroxyphenazepam in the plasma and brain of mice (5-6 animals per group) was evaluated 0.125, 0.25, 0.5, 1, 2, 3, 6, 8, 10, 24, and 48 h after intravenous (into the caudal vein) injection of drug emulsion in Twin-80 in a dose of 10 mg/kg.

The data were statistically processed using Microsoft Excel software.

RESULTS

Total radioactivity of the plasma and brain of mice after intravenous injection suggests a biphasic type of 3-HOP distribution (Fig. 1). The stationary distribution volume (V_{dis}), central compartment distribution volume (V_c), and exchange constants (Table 1) were estimated by the formulae:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t};$$
$$k_{21} = (A\alpha + B\beta)/(A+B);$$
$$k_{el} = \alpha\beta/k_{21};$$
$$k_{12} = (\alpha+\beta)/(k_{21}+k_{el});$$
$$V_c = D/(A+B);$$
$$V_{dss} = V_c(1+k_{12}/k_{21}).$$

The transdermal intake of 3-HOP was studied by comparing the areas under the concentration-time curves after intravenous ($AUC_{i/v}$) and transdermal (AUC_{trans}) administrations; bioavailability of the transdermal form was evaluated as the ratio of areas under pharmacokinetic curves after transdermal and intravenous treatment:

$$f = \frac{AUC_{trans}}{AUC_{i/v}} \times \frac{D_{i/v}}{D_{trans}},$$

where $D_{i/v}$ and D_{trans} are 3-HOP doses injected intravenously and applied transdermally, respectively.

One of 3-HOP metabolites is hydrophilic glucuronic conjugate, while free 3-HOP (more lipophilic) is presumably accumulated in the brain. At first glance, this suggests that the brain and plasma correspond to the peripheral and central compartments of the kinetic

TABLE 1. Pharmacokinetic Parameters of Intravenous and Transdermal Administration of 3-Hydroxyphenazepam (*M*±*m*)

Parameter	Plasma	Brain
A	16,416±5412	15,316±5820
α	0.2453±0.1550	0.1838±0.0700
B	1216±205	684±263
β	0.036±0.021	0.0268±0.0120
Half-distribution time, h	283.0±0.3	3.77±0.98
Half-life period, h	19.0±1.6	25.86±8.24
Stationary distribution volume, liters	84.43±17.26	111.27±13.24
Central compartment distribution volume, liters	54.1±17.28	59.61±13.25
k_{12}, h^{-1}	0.0286±0.005	0.0286±0.0053
k_{21}, h^{-1}	0.051±0.019	0.033±0.018
k_{el}, h^{-1}	0.2017±0.0200	0.1490±0.0022
$AUC_{0-\infty}$, cpm×h^2/ml	23,913±7289 / 133,097±1595	23,017±4496 / 198,542±1036
Bioavailability degree *f*	1.32±0.12	1.21±0.10

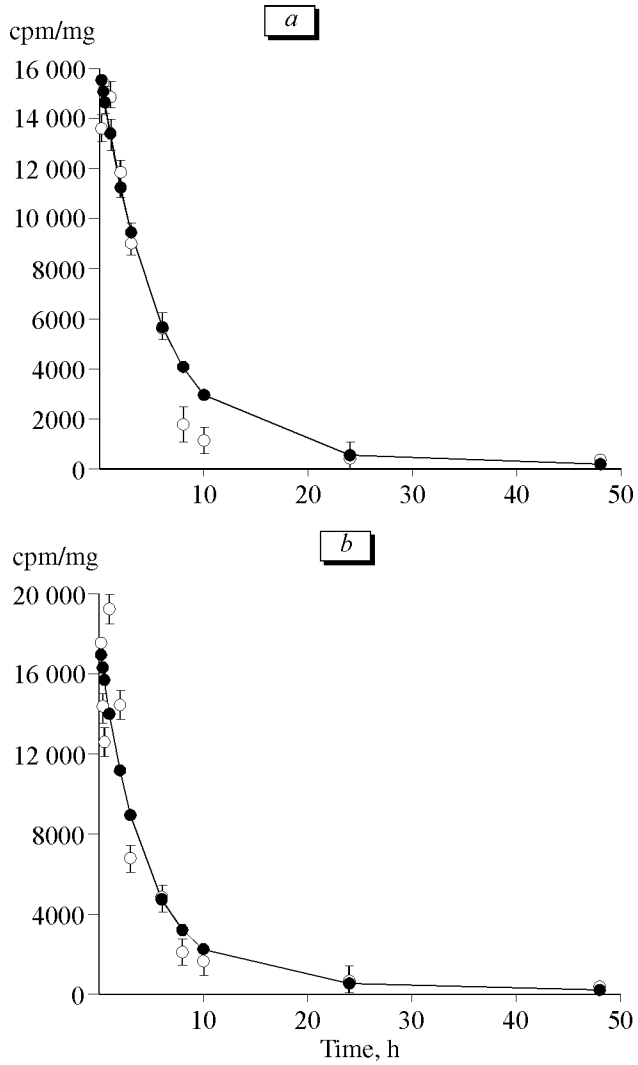

Fig. 1. Experimentally determined (points) and theoretically estimated (lines) concentrations of radioactive 3-hydroxyphenazepam metabolites in the brain (*a*) and plasma (*b*) in mice receiving intravenous injection of 3-hydroxyphenazepam (10 mg/kg).

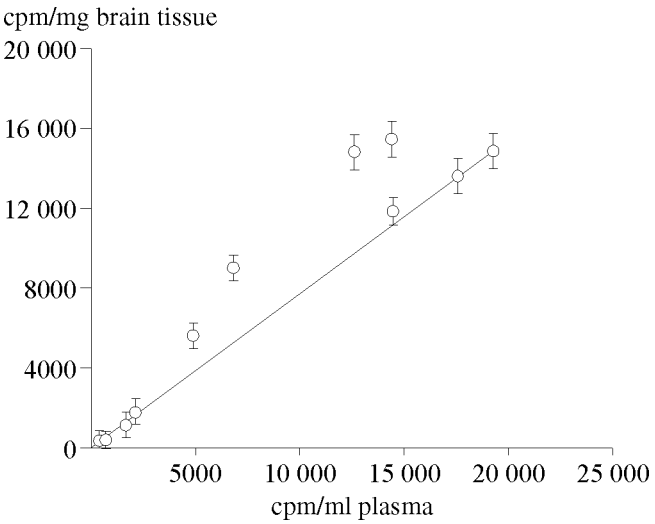

Fig. 2. Concentrations of radioactive metabolites in the brain as a function of their plasma concentration after intravenous injection of 3-hydroxyphenazepam (10 mg/kg).

scheme. In view of the absence of significant differences in drug concentrations between the plasma and brain we combined them in a common compartment (Fig. 2). Insignificance of difference of preexponential factors and constants (Table 1) also suggests that the plasma and brain as the central compartment.

TABLE 2. Anticonvulsant Effect (by Antagonism with Corazole) of Application of TTS Containing 3-HOP (*M*±*m*)

Time, h	Dose, mg/kg	
	inducing clonic tonic convulsions	inducing tonic extension
0 (control)	43.94±5.12	73.4±7.35
0.25	78.94±12.40	131.93±22.10
0.5	117.01±18.20	187.50±32.56
1	172.80±22.56	250.0±35.2
3	120.15±16.74	214.29±16.90
6	162.30±25.11	200.45±20.10
8	97.59±9.02	206.3±15.9
12	121.47±12.50	198.62±22.50
24	105.70±9.65	170.9±14.2
48	86.24±14.4	171.95±19.70

Application of the transdermal form containing 3-HOP ensured penetration of the drug into the body (Fig. 2). This process consists in two phases. Rapid phase 1 (from 0.25 to 12 h) was characterized by accumulation of total radioactivity of the plasma and brain. During slow phase 2 the concentration of radioactive products in the plasma and brain was maintained at a constant level for 24 h (24-48 h).

After application of TTS with 3-HOP the anticonvulsant effect persisted throughout the experiment (Table 2). The dynamics of this effect by the dose inducing clonic tonic convulsions and by the dose inducing tonic extension corresponded to the kinetics of radioactive products in the brain.

Bioavailability of the transdermal form of 3-HOP calculated by the method of Dost was above 1.0 (Table 1). High absolute availability attested to cumulation of radioactive 3-HOP metabolites in the body. It is known that intensive hepatointestinal circulation of 1,4-benzodiazepines and prolonged absorption of 3-HOP from the matrix determine unproportional increase in the area under the pharmacokinetic curve and overestimation of drug bioavailability.

Thus, distribution kinetics 3-HOP is described by a biphasic scheme.

Constants of exchange between compartments and drug distribution volumes were determined on the basis of total radioactivity parameters.

Bioavailability of transdermal 3-HOP is 1.32±0.12 for the plasma and 1.21±0.10 for the brain.

REFERENCES

1. S. A. Andronati, G. Ya. Avrutskii, A. V. Bogatskii, *et al.*, *Phenazepam* [in Russian], Kiev (1982).
2. N. Ya. Golovenko, I. A. Kravchenko, V. G. Zin'kovskii, *et al.*, *Byull. Eksp. Biol. Med.*, **130**, No. 12, 633-635 (2000).
3. I. A. Kravchenko, V. G. Zin'kovskii, A. I. Aleksandrova, and E. S. Kashirkina, *Visnik Farmatsii*, **20**, No. 2, 127-129 (1999).
4. *Drug Technology and Standardization* [in Russian], Kharkov (1996).
5. B. Berner and V. A. John, *Clin. Pharmacokinet.*, **26**, No. 2, 121-134 (1994).
6. D. A. Edwards and R. Langer, *J. Pharm. Sci.*, **83**, No. 9, 1315-1334 (1994).
7. V. V. Ranade, *J. Clin. Pharmacol.*, **31**, No. 5, 401-418 (1991).