Experimental Study of the Pharmacokinetics of a Tryptophan-Containing Dipeptide GB-115

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Pharmacokinetics of an original compound GB-115 (N-phenylhexanoylglycyltryptophan) synthesized on the basis of the structure of endogenous tetrapeptide cholecystokinin-4 was studied by means of high-performance liquid chromatography. Pharmacokinetic parameters were calculated after intravenous and peroral administration of GB-115. Our results indicate that this dipeptide is resistant to peptidases. The absolute bioavailability of GB-115 is 4.65%.

Key Words: *pharmacokinetics; dipeptide; anxiolytic; cholecystokinin; receptors*

Anxiolytic activity of cholecystokinin is associated with the presence of the C-terminal fragment tetrapeptide Trp-Met-Asp-Phe-NH_2 and manifested due to interaction with cholecystokinin-2 receptors [3,5]. Preclinical and clinical trials revealed several limitations of cholecystokinin-2 receptor antagonists, in particular their low bioavailability due to insufficient absorption in the gastrointestinal tract (GIT) and rapid elimination in the liver [4]. Substituted dipeptides, potential anxiolytics, were synthesized at the V. V. Zakusov Institute of Pharmacology (Prof. T. A. Gudasheva); of them N-phenylhexanoylglycyltryptophan (GB-115) was most active. Previous studies showed that intraperitoneal and peroral administration of this compound in doses of 0.05-0.20 mg/kg produced an anxiolytic effect [2].

Here we studied pharmacokinetics of GB-115 after intravenous and peroral administration to rats.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 200±20 g and obtained from Stolbovaya nursery. The animals were kept in a vivarium (10 rats per cage) under natural 12:12-h light/dark conditions and fed a standard diet. Aqueous solution of GB-115 and Tween 80 was injected intravenously or given perorally (10 and 50 mg/kg, respectively). The animals (n=6) were decapitated 5, 10, 15, 30, 45, and 60 min after dipeptide administration. The blood was collected in heparinized tubes. The plasma was obtained by centrifugation at 3000 rpm for 10 min.

GB-115 was extracted with a 10-fold volume of chloroform at neutral pH and with constant sha-

TABLE 1. Pharmacokinetics of GB-115 in Rat Plasma after Intravenous and Peroral Administration ($M\pm m$)

Time, min	GB-115 concentration, μg/ml	
	intravenous injection	peroral administration
5	1.110±0.015	0.160±0.015
10	0.820±0.010	0.200±0.014
15	0.650±0.062	0.250±0.018
30	0.380±0.042	0.140±0.016
45	0.230±0.030	0.090±0.036
60	0.160±0.025	0.056±0.022

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TABLE 2. Pharmacokinetic Parameters of GB-115 after Intravenous and Peroral Administration

Parameter	Intravenous injection	Peroral administration
Maximum concentration, μg/h	1.11	0.248
Time of ataining maximum concentration, h	—	0.25
Elimination constant, h^{-1}	2.086	1.972
Elimination half-life, h	0.332	0.352
Mean retention time of unchanged peptide, h	0.349	0.396
Plasma clearance, liters/h	13.89	305.06
Distribution volume, liters	4.7	154.7
Mean absorption time, h	—	0.247
Area under pharmacokinetic curve, μg/ml/h	0.740	0.169
Absolute bioavailability, %	—	4.648

king over 15 min. For better separation of the organic and aqueous phases the samples were centrifuged at 5000 rpm for 5 min. Chloroform extracts were collected and dried under nitrogen flow. Dry residues were diluted in the eluate and analyzed by chromatography in a Perkin Elmer computer system equipped with an UV/VIS LC-290 spectrophotometric detector and LC-250 isocratic pump. Chromatographic separation was performed on a Phenamenex Luna 5 μ C_{18} column [2]. The system of potassium orthophosphate (0.1 M, pH 3.2) and acetonitrile (60:40 v/v) served as the eluate. Quantitation was performed by the method of absolute calibration. The calibration curve was linear at concentrations of 50-1000 ng/ml. The correlation coefficient was 0.99878. The main pharmacokinetic parameters were calculated by the model-independent method of statistic moments [1].

RESULTS

The concentration of GB-115 in rat plasma peaked 5 min after intravenous injection (1.1 μg/ml, Table 1) and then progressively decreased to 0.16 μg/ml over the next 1 h. After peroral administration, the dipeptide was rapidly absorbed in GIT. The concentration of GB-115 reached maximum 15 min after peroral administration and over the next 1 h progressively decreased to 0.056 μg/ml, similarly to experiments with intravenous injection.

Some pharmacokinetic parameters of substance GB-115, including elimination constant, elimination half-time, and mean retention time of unchanged peptide, did not depend on the administration route (Table 2). GB-115 was characterized by longer elimination half-time compared to some natural neuropeptides. For example, elimination half-time for thyroliberin and enkephalins in rat plasma after intravenous injection is 2-5 min. Our results indicate that GB-115 is resistant to enzymatic effect of peptidases. At the same time, plasma clearance and distribution volume of GB-115 after peroral administration surpassed the corresponding parameters after intravenous injection. These differences are related to greater distribution in internal organs and more intensive metabolism during the first passage through the liver after peroral administration of GB-115. The area under the pharmacokinetic curve after peroral administration was much lower than after intravenous injection. These data reflect not only rapid elimination of the dipeptide with enzymes in the liver and GIT, but also incomplete absorption of GB-115 after peroral administration. The absolute bioavailability of GB-115 was 4.65%. Hence, this dipeptide is characterized by high bioavailability.

Our results indicate that new dipeptide compound GB-115 with selective anxiolytic properties is characterized by a longer half-life compared to natural neuropeptides. Therefore, this dipeptide is resistant to enzyme systems of blood plasma and GIT and is characterized by high absolute bioavailability in rats (relative to peptide compounds). Pharmacokinetic study showed that GB-115 can be recommended as a preparation for peroral administration.

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