PHARMACOLOGY AND TOXICOLOGY

Pharmacokinetics of New Nootropic Acylprolyldipeptide and Its Penetration across the Blood-Brain Barrier after Oral Administration

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Pharmacokinetics of GVS-111, a new acylprolyldipeptide with nootropic properties and its penetration across the blood-brain barrier were studied in rats using HPLC. It was found that the dipeptide is absorbed in the gastrointestinal tract, enters the circulation, and penetrates through the blood-brain barrier in an umodified state.

Key Words: *acylprolyldipeptide; GVS-111; pharmacokinetics; blood-brain barrier permeability*

There is a growing interest in the therapeutic use of natural peptides, its fragments, analogs, and synthetic medicinal peptides. Principal characteristics of these preparations are their high specificity when used in low doses, the absence of side effects, and low toxicity.

Oral administration is more preferable for long-term treatment with natural peptides, but it reduces peptide bioavailability and is associated with high variability in their plasma concentration due to rapid enzymatic degradation. The discovery of mixed peptidase activity in the intestinal mucosa led to firm belief that peptides are rapidly hydrolyzed by intestinal cells and absorbed only as free amino acids [2,9,10]. Enzyme barriers limiting peptide absorption in the gastrointestinal tract (GIT) are presented by peptidases located in the intestinal villi and enterocyte cytosol [6,7,10,13].

There are ample experimental and clinical data showing that oligopeptides, especially di- and tripeptides produce their effects after oral administration. The most hydrolysis-resistant are di- and tripeptides containing glycine, proline, hydroxyproline, and D-amino acids; these peptides can be absorbed and enter unchanged the liver portal vein [5,9,11]. N-Acylation of amino acid residues increases peptidase resistance by decreasing the number of hydrogen bonds and increasing their lipophilicity. Lipophilicity of short peptides predicts their ability to penetrate across the blood-brain barrier (BBB) [3,4,10]. Apart from passive absorption, di- and tripeptides are transported across the bruch border membranes via active ATP-dependent mechanism against the concentration gradient [2,6-8,12,14].

While developing medicinal preparations on the basis of protected prolylglycine, the Institute of Pharmacology of the Russian Academy of Medical Sciences came out with a highly efficient acylprolyldipeptide with nootropic properties, GVS-111 (N-phenylacetyl-L-prolyl-glycine ethyl ester). This dipeptide shows much higher potency and activity than standard nootropic piracetam [13].

We previously found that GVS-111 is more resistant to enzymatic degradation in parenteral administration than natural neuropeptides [1].

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This study was aimed at investigation of GVS-111 pharmacokinetics and its ability to penetrate across BBB after oral administration.

MATERIALS AND METHODS

Experiments were carried out on outbred albino male rats weighing 200-220 g. GVS-111 was administered *per os* as a water solution in a dose of 50 mg/kg. The rats were decapitated 5, 10, 15, 20, and 25 min after administration (5 animals per point). Plasma was obtained by 10-min centrifugation of heparinized blood at 3000 rpm. The brain was rapidly removed, washed with cold saline, dried with filter paper, weighed, minced with scissors, and homogenized in a glass homogenizer with 3 volumes of distilled water. Plasma samples and brain homogenates were diluted by 5 volumes of diethyl ether and shaken for 10 min to remove coextractive compounds. After separation of organic and water phases, the water layer was shaken for 10 min with 5 volumes of chloroform in a special shaker and centrifuged for 5 min at 8000 rpm. The chloroform extracts were dried in a rotor evaporator at room temperature, dissolved in 200 μl ethanol, and analyzed by HPLC.

The content of GVS-111 in blood and brain samples was determined by HPLC with a Shimadzy RF-535 spectrofluorescent detector at 250 and 290 nm excitation and emission wavelengths, respectively. Chromatographic separation was performed at 22°C on a Zorbax CN column (250×4.5 mm, 5 μm beads) with a 50×4.6 mm Silasorb-C_{18} precolumn. An acetonitrile:water:glacial acetic acid mixture (25:75:0.1 v/v) was used as a principal solvent. The elution time for GVS-111 was 9.8 min at a flow rate of 1 ml/min. Two additional solvents were used to identify GVS-111 more precisely: acetonitrile:water (30:70) and acetonitrile:water (25:70). Under these conditions the elution time at a flow rate of 1 ml/min was 7 and 8.5 min, respectively.

The content of GVS-111 in plasma and brain samples was calculated using a Shimadzy C-R1A integrator, which automatically recorded the elution time and computed the peak areas of the test substance. The data were analyzed with Student's *t* test. Principal pharmacokinetic parameters were calculated from these data with the help of Model Independent software.

RESULTS

The chromatograms of blood and brain homogenate samples obtained with 3 different solvents showed a peak with the elution time corresponding to the standard (pure GVS-111), which allowed to identify the substance as GVS-111.

GVS-111 was rapidly absorbed in GIT and entered the circulation: after 5 min it was found in the plasma, its concentration peaked 10 min after administration, then rapidly decreased, and after 25 min no GVS-111 was detected (Fig. 1).

The comparison of these data with the results of parenteral administration [1] showed the same pharmacokinetics of GVS-111 irrespective of the administration route: the absorption stage after oral administration was very short and the plasma concentration of the drug was close to that after parenteral administration. Similar plasma level of GVS-111 was observed after 5 mg/kg parenteral and 50 mg/kg oral administration. The dynamics of GVS-111 elimination after oral and parenteral administration was also similar [1].

Considerable differences related to the administration route were revealed in GVS-111 metabolism. After parenteral administration, apart from the unchanged GVS-111 molecule, the plasma contained 2 phenylacetyl metabolites phenylacetylproline and phenylacetic acid, which were not detected after oral administration. At the same time, plasma chromatograms after oral administration showed a peak which was eluted before GVS-111 and differed from the known phenylacetyl-derivatives by the elution time. It could be either hydroxylated or methylated GVS-111 metabolite. This difference in GVS-111 metabolism in different administration routes seems to be due to the fact

TABLE 1. Pharmacokinetic Parameters of GVS-111 after Oral Administration

Parameters	Plasma	Brain
Area under pharmacokinetic curve	0.216 μg/ml×h	0.297 μg/ml×h
Elimination constant, h^{-1}	5.980	6.032
$T_{1/2}$, h	0.116	0.115
Mean lifetime, h	0.256	0.280
Plasma clearance, liter/h	231.7	160.4
Distribution volume, liter	38.75	26.60
Time to peak concentration, h	10	10
Peak concentration	0.820 μg/ml	1.289 μg/ml

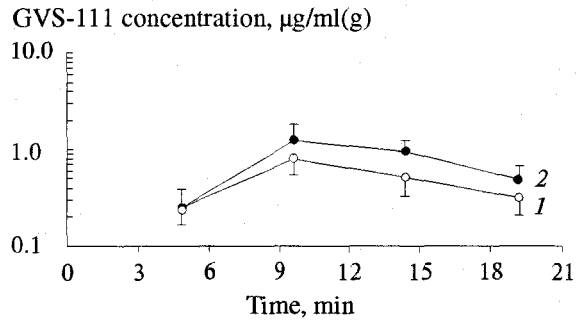

Fig. 1. Pharmacokinetics of GVS-111 in rat plasma (*1*) and brain tissue (*2*) after oral administration.

that parenterally administered peptide is hydrolyzed preferentially by plasma peptidases, while metabolic transformations after oral administration occur during its first passage through the liver.

GVS-111 rapidly permeated BBB. Its brain and plasma kinetics were very similar: brain concentration of GVS-111 also peaked after 10 min, but its content was higher than in the plasma (Fig. 1). The brain/plasma ratio exceeded 1 at all terms after attaining the peak concentration, which attests to high tropism of the peptide to the brain tissue and its high specific bioavailability. The permeation of unchanged GVS-111 into the brain after oral administration can be due to high lipophilicity of this molecule. Dipeptide permeation across BBB can be predicted by its lipophilicity. At the same time, active transport of GVS-111 across BBB membranes cannot be excluded.

The calculated pharmacokinetic parameters of GVS-111 for plasma and brain tissue were similar (Table 1). These data suggest slower peptide elimination from the brain compared to the plasma.

Therefore, this study showed that orally administered GVS-111 is absorbed by GIT, enters the circulation in an unchanged form, and penetrates across BBB.

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