NOOPEPT PHARMACOKINETICS AFTER INTRAVENOUS ADMINISTRATION OF A LYOPHILIZED MEDICINAL FORM IN RATS

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The pharmacokinetics of noopept, a dipeptide drug possessing nootropic and neuroprotective properties, were evaluated in rats after the intravenous administration of its novel formulation for injections prepared using lyophilization in the presence of mannitol. The pharmacokinetic characteristics of the new preparation are comparable with those of aqueous solutions of the parent substance and can be recommended for further investigation and implementation into medical practice.

Investigations showed that the new dipeptide drug noopept (N-phenylacetyl-*L*-prolylglycine ethyl ester) possesses pronounced nootrope (cognition enhancer) and neurioprotector properties [1]. As is known, modern medicine exhibits considerable demand for such agents, which is related to the high incidence of disorders (stroke, cerebrocranial traumas, other brain pathologies) accompanied by violation of cognitive functions. Emergency aid in the acute period of such disorders implies the use of effective drugs in the form of injections. However, noopept is subject to hydrolysis in the course of long-term storage of aqueous solutions, which hinders the administration of this drug by injection in cases of emergency. For this reason, a new noopept formulation for injections has been developed using the method of lyophilization in the presence of mannitol (an auxiliary substance) at a mannitol/noopept ratio of 1 : 10 (w/w). The results of a preliminary pharmacological investigation of this ready-to-use medicinal form of noopept in rats with a model of ischemic stroke caused by distal occlusion of the medial cerebral artery showed a reliable decrease in the extent of cerebral cortex damage in operated animals treated with noopept in a dose of 0.5 mg/kg 15 min and 2, 24, and 48 h after occlusion [2].

Previously, we have studied the pharmacokinetics of noopept in rats upon intravenous injection of an aqueous solution of the parent substance in a dose of 5 mg/kg [3]. Taking into account that lyophilization increases the stability of noopept, while the presence of mannitol can significantly modify physicochemical properties of the drug – in particular, increase its solubility in water [4, 5] – it was not excluded that these factors could also change the pharmacokinetics and distribution of noopept in the organism.

The study was aimed at determining the pharmacokinetics of noopept upon single intraperitoneal injection of a solution prepared from the new lyophilized formulation in comparison to the parent substance.

EXPERIMENTAL PART

The experiments were performed on white mongrel male rats weighing 180 – 220 g.

A solution for injections was prepared *ex tempore* by dissolving a lyophilized drug in 1 ml of distilled water and injected into the tail vein in a dose of 10 mg/kg. The animals were decapitated with discrete time intervals after treatment (5, 10, 15, 20, 25, and 30 min) and the blood for analyses was collected in heparin-treated tubes.

The samples of plasma (2 ml, obtained by centrifuging whole blood for 10 min at 3000 rpm) were doubly extracted with 10-fold volumes of chloroform for 15 min on an electric shaker. The extracts were combined and the solvent was evaporated in airflow. The dry residue was dissolved in 0.5 – 1.0 ml of the mobile phase, and 200 μl of this solution was introduced into the injection loop of a chromatographic system.

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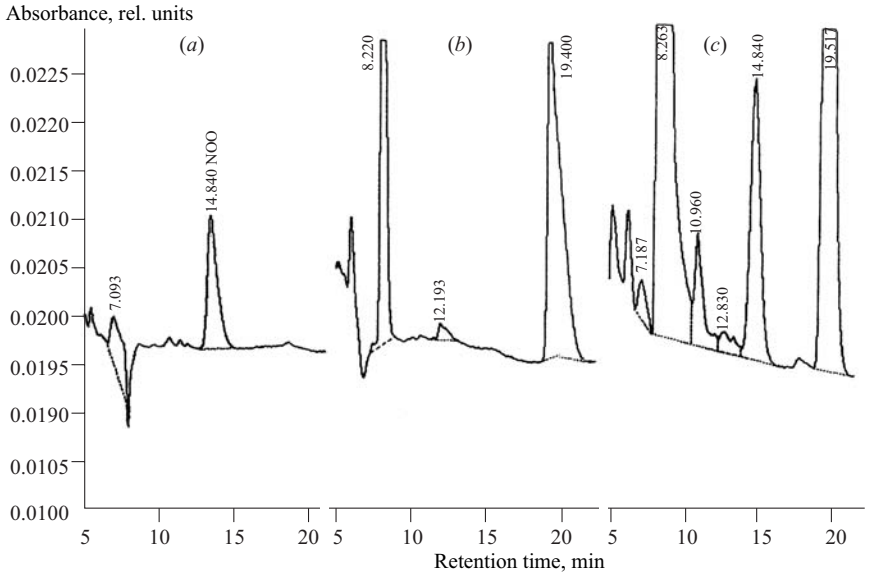

Fig. 1. Typical chromatograms of (a) reference aqueous noopept solution, (b) control sample of blood plasma after double extraction with chloroform and (c) solution of noopept extracted from plasma taken 5 min after injection of a solution of the new lyophilized preparation.

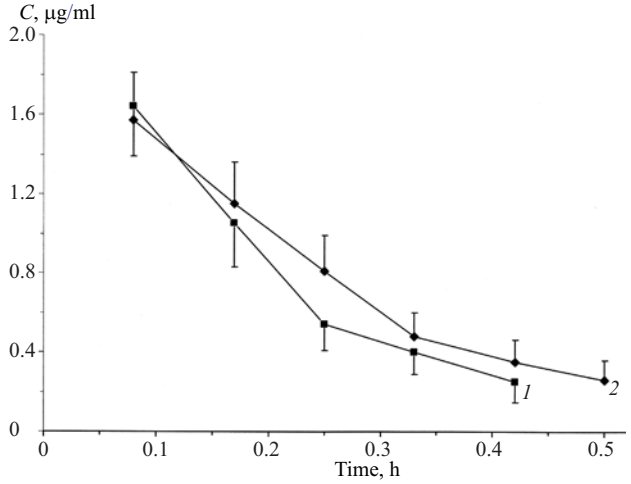

Fig. 2. Plots of noopept concentration in the blood plasma (C) versus time upon administration in rats: (*1*) parent substance; (*2*) new lyophilized formulation.

The quantitative determination of noopept in the blood plasma was performed using high-performance liquid chromatography (HPLC) on a computer-controlled Perkin-Elmer system (USA) equipped with an isocratic pump (LC-250) and a variable-wavelength UV detector (LC-290) tuned to 206 nm. The HPLC measurements were performed at room temperature in the following regime: column, 4.6 × 250 mm Luna (Phenomenex); reversed-phase sorbent C 18(2), 5 μm; mobile phase, methanol – ammonium monosubstituted phosphate buffer, 0.1 M pH 4.6 (40 : 60, v/v); flow rate, 1.0 ml/min. The retention time of noopept under these conditions is 15 min. The detection threshold for noopept was 40 ng/ml.

TABLE 1. Pharmacokinetic Parameters of Noopept in Rats upon Intravenous Single Injection (10 mg/kg, i.p.) of Solutions Prepared from the New Lyophilized Formulation and a Standard Parent Substance

Parameter	Lyophilized preparation	Parent substance
C_0, μg/ml	13.46	15.34
K_{el}, h^{-1}	4.427	4.877
$T_{1/2}$, h	0.157	0.142
MRT, h	0.117	0.097
AUC, μg/(ml h)	0.965	1.018
V_d, ml	1200	957
Clp, ml/h	10370	9822

The content of noopept in chromatographic fractions was determined by the method of direct absolute calibration with respect to the area under peaks. The calibration graph was linear in a range of concentrations from 50 to 1000 ng/ml. The percentage extraction of noopept from the blood plasma of rats was 95.8 ± 1.46%. The main parameters of pharmacokinetics were calculated within the framework of a model-independent approach using the corresponding program package. The results were statistically processed using Statistica (Ver. 6) software.

RESULTS AND DISCUSSION

As can be seen from the typical chromatograms presented in Fig. 1, the retention time of noopept chromatographed in an aqueous solution is about 15 min.

Figure 2 shows the kinetics of noopept in the blood plasma of rats upon a single intravenous injection of a solu-

tion prepared from the new lyophilized preparation, in comparison to the aqueous solution of the parent substance (also injected in a dose of 10 mg/kg). As can be seen, the two kinetic profiles are very close to each other and have generally the same shapes.

The observed pharmacokinetics was characterized by a set of parameters calculated using the method of statistical moments (Table 1), including the initial concentration (C_0), the elimination rate constant (K_{el}), the area under the pharmacokinetic (concentration versus time) curve (AUC), the half-elimination time ($T_{1/2}$), the mean retention time (MRT) of the drug in the organism, the distribution volume (V_d), and the plasma clearance (Clp).

As can be seen from the data presented in Table 1, the parameters of noopept pharmacokinetics are virtually the same for the drug solutions prepared from the new lyophilized form and the standard parent substance. Therefore, the results of our experiments show that the new formulation is not inferior to the parent substance and, hence, can be recommended for further investigation and implementation into medical practice.

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