



Study of pharmacokinetic of new peptide drug 1-deamino-arginine-vasotocin for hypernatremia correction

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Abstract

Introduction: The pharmacokinetics studies are some of the necessary parts of the drugs preclinical investigations. Pharmacokinetic properties of new peptide drug 1-deamino-arginine-vasotocin (dAVT) in the form of an injection solution for intravenous and intramuscular administration for hypernatremia correction were investigated.

Materials and methods: The study was carried out on male rats and rabbits with a single intravenous administration of the drug in three doses, a single intramuscular administration in one dose and multiple administration to rats in one dose. To determine natriuretic peptide concentration in blood plasma, tissues, and excretes, assays based on a sodium level change measurement using a biochemical analyzer have been developed and validated. Pharmacokinetic parameters were calculated by the model-independent method of statistical moments.

Results and discussion: The pharmacokinetics of the drug was found to be linear after a single administration of dAVT drug in the dose range 3–10 µg/kg for rats and rabbits. The relative bioavailability of dAVT after intramuscular and intravenous administrations was more than 30%. After a biomarker content change, the active substance was intensively distributed into highly vascularized organs (spleen), the organs that provide metabolism and subsequent excretion (liver and kidneys), whereas it hardly reached moderately and weakly vascularized tissues (muscles, omentum). Less than 10% dAVT was excreted with urine; no dAVT was determined in feces; and repeated administration did not lead to its cumulation.

Conclusion: Pharmacokinetics parameters of new nonapeptide drug 1-deamino-arginine-vasotocin were evaluated after original analytical biomarker approach. The study included all main areas necessary to characterize the original drug pharmacokinetic.

Keywords

1-deamino-arginine-vasotocin, solution for injection, parenteral administration, pharmacokinetics, rats, rabbits, plasma, sodium level.

Introduction

Hypernatremia (more than 145 mmol/L) occurs not only under extreme physiological conditions (Kutina et al. 2005), but also accompanies some serious pathological states (Natochin et al. 1996; Kutina et al. 2005; Sheyman 2019). Acute hypernatremia can cause demyelination in the brain. Clinical manifestations of demyelination may include seizures, behavioral disorders and motor disorders, in serious cases – paralysis, speech and swallowing disorders due to demyelination in brain structures. But even serious neurological disorders during osmotic demyelination are most often reversible. Chronic hypernatremia causes reversible encephalopathy. Even a moderate increase in the blood sodium concentration above the normal values increases the risk of death, but neurological complications rarely cause death. Diuretic dehydration therapy in patients with impaired consciousness (for strokes, traumatic brain injuries, etc.) is accompanied by a high risk of hypernatremia due to the lack of thirst and inadequate water consumption.

Thus, it is necessary to search for drugs to reduce the volume of extracellular fluid, increase the excretion of sodium ions by the kidneys, but to increase the reabsorption of osmotically free water in the renal tubules.

Rapid progress in the synthesis of biologically active peptides has provided a basis for chemically produced compounds with a selective biological activity, significant stability and duration of action. Natriuretic nonapeptide (deamino-cysteinyl-tyrosyl-isoleucyl-glutamyl-asparaginyl-cysteinyl-arginyl-glutamylamine, 1-deamino-arginine-vasotocin, dAVT) is a synthetic peptide with an the ability to correct hypernatremia (Natochin et al. 2007; Natochin and Kutina 2009; Karlina et al. 2016).

The dAVT mechanism of action is based on inhibition of sodium ion reabsorption in distal nephron segment and an increase in water re-absorption in collection tubes due to an interaction with V1a- and V2-receptors of vasopressin, which causes increased excretion of sodium ions by the kidney while preserving water in body (Natochin et al. 2007; Natochin and Kutina 2009; Karlina et al. 2016). It is promising to create a drug to correct hypernatremia by injecting this active substance (Karlina et al. 2016).

The study of pharmacokinetics, including assessment of kinetics of absorption, distribution and elimination of pharmacological agents in the organism, is a necessary part of preclinical studies of the original drug required for its registration and is regulated by the official regulatory documents (SCEEMP 2012).

The purpose of the present study was to evaluate pharmacokinetic properties of a new peptide drug for correction of hypernatremia for parenteral use (dAVT) in two animal species – rats and rabbits.

Materials and methods

Drug product under study

dAVT in form of solution for injections, 12 µg/ml, developed and produced at St. Petersburg Institute of Pharmacy (Russia) according to the original technology (Karlina et al. 2016) by using deamino-cysteinyl-tyrosyl-isoleucyl-glutamyl-asparaginyl-cysteinyl-prolyl-arginyl-glutamylamine (CJSC Innovation Research and Production Center PEPTOGEN, Russia) was the object of the study.

Experimental animals and design

A pharmacokinetics study (in vivo experiments) was carried out on male rabbits and male rats (RMC HOME OF PHARMACY, Russia). The experiments were performed in accordance with the methodological guidelines (SCEEMP 2012, 2013) and regulatory documents (The Laboratory Practice Rules for Preclinical Studies in the Russian Federation (GOST 33044-2014); directive 2010/63/EU of the European Parliament and Council of the European Union of 22.09.2010 on Protection of Animal Used in the Scientific Purposes, etc.) for preclinical studies using laboratory animals; the experiments were also approved at a session of the Bioethical Committee (No. 3.34/17 of 07.06.2017).

The drug was administered to the rats intravenously once in the doses of 3, 5 and 10 µg/kg and repeatedly in a dose of 5 µg/kg (once a day for 3 days), to the rabbits – intravenously once in the doses of 3, 5 and 10 µg/kg. Additionally, dAVT substance (as a solution in water for injections with concentration of 12.5 µg/ml) was administered intramuscularly once in a dose of 5 µg/kg to determine the relative bioavailability in rats and rabbits.

The distribution of the animals into experimental groups was performed by modified block randomization (Bland 2000). For the pilot experiment, the rats were distributed into 1 group containing 15 animals, later divided into 5 subgroups by the number of time points, with 3 animals in each subgroup. For the main experiment, the rats were divided into 6 groups: 4 groups of 6 animals each and 1 group of 50 animals (this group was further divided into 10 subgroups by the number of time points, with 5 animals in each subgroup because euthanasia was necessary for sample collecting); the rabbits were divided into 4 groups of 6 animals each.

Blood samples from rats and rabbits were taken 0 (before administration), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 and 24 h after the administration; in the pilot experiment in the rats, the time points were 0, 0.25, 1, 2 and 6 h, with heparin used as an anticoagulant. The blood samples were collected from the rat tail vein or from the rabbit marginal ear vein, through an intravenous catheter (22G, KD Medical GmbH Hospital Products, Germany) in the volume of 0.3 ml for rats or 2.0 ml for rabbits at each time

point. For the rats in the pilot experiment and in the main experiment after a single intravenous administration in a dose of 5 µg/kg, the blood samples were collected from the heart during euthanasia in a CO₂ camera simultaneously with sampling tissues in the volume of 2.0 ml at each time point. The samples were centrifuged to obtain blood plasma (15 min, 3000 rpm), with the resulting plasma frozen and stored at -20 °C.

Five tissues (from liver, kidneys, spleen, muscles, omentum), as well as two types of excreted (urine and feces, at 5 time intervals, sampling was performed using metabolic cells) were selected to assess the distribution of the drug in tissues, as well as its excretion, when administered to the rats once in a dose 5 µg/kg (at ten time points, along with blood sampling, the manipulation was accompanied by euthanasia of the animals). The obtained samples of tissues and feces were placed in grippers, weighed and frozen. Volume was measured for the urine samples, and samples were also frozen. All the samples were thawed prior to an analysis.

Biosamples analysis

A technique for analyzing the active drug substance (dAVT) concentration in blood plasma was developed and validated. Due to the impossibility of a direct analysis of the active substance molecule, indirect determination using a biomarker (sodium ion concentration) was used. The assay was based on the sodium ion determination by a biochemical analyzer. The procedure was adapted and revalidated for an analysis of rat tissues (using the liver and kidneys).

dAVT solutions in 0.9% potassium chloride solution with concentrations of 100; 40.0; 20.0; 4.0; 2.0; 0.8; 0.27; 0.16 µg/ml were used to prepare model mixtures with biomaterial and to perform necessary validation tests. Calibration model mixtures with whole blood of the intact animals (rats and rabbits), as well as homogenates of rat tissues (liver and kidneys), were prepared to construct a calibration curve for the dAVT concentration versus the change in sodium concentration in the biomaterial. Briefly, 540 µl of whole animal blood, 60 µl of dAVT solutions with different concentration were added to the cells of the 24-well plate (to obtain a calibration sample with a dAVT concentration of 0 µg/ml (blank sample), 60 µl of 0.9% sodium chloride solution was added to 540 µl of whole blood); the samples in the plate were incubated for 30 min at 350 rpm at 37 °C, transferred to Eppendorf centrifugal tubes, centrifuged at 3000 rpm for 15 min, and the resulting blood plasma was collected. The sodium concentration (mmol/L) in these plasma samples was determined by a biochemical analyzer. The change in sodium concentration (ratio) was calculated, and the calibration curve was constructed. In the analysis of the experimental samples, the same procedure was used, except for the stage of adding spikes.

To obtain a tissue homogenate of an organ (liver, kidneys, spleen, omentum), the organ was placed in

a plastic tube, weighed, washed with purified water, homogenized with a Polytron PT-MR 1600E tissue grinder (Kinematica AG, Switzerland) with purified water in mass: volume ratio 1:9.5. To 1 ml of the resulting homogenate, 1 ml of purified water was added; the sample was vortexed for 30 seconds, then centrifuged at 6000 rpm for 15 minutes; an aliquot was taken to determine sodium concentration by a biochemical analyzer. When treating muscles, an additional stage of triturating with glass sand was used.

Sodium concentration was determined on biochemical automatic analyzers using ready, commercially available reagent kits. A plasma analysis was performed on a biochemical automatic analyzer A-25 (Biosystems, Spain), and a tissue homogenates analysis – on an Accent 200 analyzer (Cormay, Poland). The determination principle is based on the enzymatic pseudokinetic (two-point) method (for automatic analyzers) with the optical density measurement at a wavelength of 420 (405–436) nm. Sodium ion activated Na-dependent β-galactosidase. The activated enzyme split o-nitrophenyl-β-D-galactopyranoside (ONPG) into galactose and colored o-nitrophenol. The rate of splitting ONPG is proportional to the sodium concentration in the sample. Linearity ranged within the concentrations of 100–160 mmol/l, with deviation of under 5%.

To calculate the concentration of dAVT in blood plasma and other types of the biomaterial, the parameter $\Delta = C_{Na(n)}/C_{Na(0)}$ was determined for each sample, where Δ is the sodium concentration change (ratio); $C_{Na(n)}$ is the sodium concentration obtained for the biomaterial after administration of dAVT, mmol/L; $C_{Na(0)}$ is sodium concentration obtained for the biomaterial prior to administration of dAVT (first blood sampling point, 0 min, dAVT concentration of 0 µg/ml), mmol/L. A calibration curve was then constructed to show the correlation between Δ , obtained for the model mixtures of the biomaterial with dAVT solutions and the concentration of dAVT, µg/ml or µg/g (type $y = ax + b$). The obtained regression equations were used to calculate the dAVT content in the samples (X, µg/ml; for organs – µg/g of tissue), taking into account dilution factors of the analyzed samples.

Statistical analysis

The pharmacokinetics parameters were calculated by a model-independent method of statistical moments (Zhang et al. 2010), using the PKSolver application for Microsoft Office Excel; mean arithmetic values (\bar{X}), their corresponding standard deviations (SD), standard errors of the mean value ($S\bar{x}$) were calculated. For statistical estimation of the differences between pharmacokinetic parameters, a one-way ANOVA analysis for more than two groups or two-tailed t-test for the mean values (for two groups) were performed; the assessment was carried out at 95% confidence level with parametric or non-parametric criteria depending on normality of

data distribution. A statistical analysis of the results was performed using Microsoft Office Excel 2007 and GraphPad Prism 9 (GraphPad Software, USA).

Results and discussion

The study suggested very low doses of the active ingredient (3–10 µg/kg). Methods with high sensitivity and specificity, such as ELISA and HPLC, need to be used to detect such an amount of target analyte in the biomaterial. Based on the preliminary experiments, it was found that HPLC methods with various detection techniques (UV-, mass-), as well as ready-to-use ELISA kits to determine nonapeptide-related compounds (vasopressin, oxytocin, and desmopressin) are not suitable for the study purpose.

Therefore, an approach was tested which related to the search for and use of a biological marker (biomarker) the level of which is associated with the biological activity of the studied drug and its pharmacodynamic properties. Such an approach has been most developed for low molecular weight heparins (EMA 2017) and have been developed and successfully used to study the pharmacokinetics of drugs of natural origin (Kosman et al. 2021).

The mechanism of nonapeptide action is associated with increased renal excretion of sodium salts and intensification of selective reabsorption of water (the solvent) from the renal tubules into blood (Natochin et al. 2007; Natochin and Kutina 2009). After intramuscular administration of dAVT at a dose of 0.05 µg/100 g to the rats, excretion of sodium cations within 2 hours of the experiment increased about 20 times as compared to the control group, and reabsorption increased about 3.5 times (Natochin et al. 2007; Natochin and Kutina 2009). Change in the concentration of sodium and potassium ions may be caused by a change in the activity of Na/K-dependent adenosinetriphosphatase (ATP-ase) after administration of various drugs (Boldyrev 2008). Therefore, to select a possible biomarker, the effect of dAVT on the activity of the enzyme of Na/K-dependent ATPase and a change in sodium concentration in vitro was studied, using rats' and rabbits' whole blood and tissue homogenates (liver and kidneys). After incubation of the biomaterial with different concentrations of dAVT, the Na⁺/K⁺-ATP-ase activity was determined by the procedure by (Kashkin et al. 2002), and the sodium concentration in animals' plasma was measured using a biochemical analyzer. A dose-dependent change in the ATP-ase activity and a change in the sodium concentration were obtained for a fairly wide range of concentrations: 0.016–10 µg/ml. Na-concentration assay is more simple and automated in comparison with ATP-ase assay; due to these advantages, this parameter was selected for further analysis. Linear dependence of lgC=f(ΔNa) was obtained for model mixtures of dAVT with rats' blood (as an example) within the concentration range of 0.016–2.0 µg/ml (Fig. 1); the correlation coefficient (r) was above 0.99, indicating a high correlation between the concentration of the

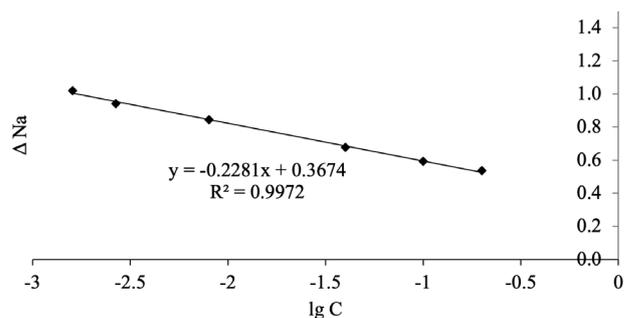


Figure 1. Correlation between change in sodium concentration (Δ Na) and logarithm of 1-deamino-arginine-vasotocin concentrations (lg C) in rat blood plasma in vitro.

detectable compound in the analyzed solution and the analytical response (Δ Na).

The detected correlation between the dAVT concentration and a change in the sodium concentration in the biomaterial was used to develop methods for target tested compound assay in laboratory animals' blood plasma and tissues. The dAVT assays in laboratory animals' blood plasma, organs and tissues (in terms of liver and kidneys) were properly validated according to the official recommendations (ICH 1995; ICH 1996; EMA 2011; FDA 2018). The main validation parameters are presented in Table 1. Satisfactory results were obtained for all the required parameters.

The developed and validated assays were used to analyze the biosamples obtained during the biological experiment. After the pilot study, the proposed assays were confirmed applicable for quantification of the target analyte in biosamples after drug administration; the total period of observation and sampling design were optimized, and the number of experimental animals was minimized. Because small sample volume was enough for the bioanalytical assay, it became possible to obtain blood samples not after euthanasia, but from rat tail vein, keeping animals alive and returning them to the stock population after the experiment. These results demonstrate the extra importance of the pilot study in a bioethical study design and analytical aspects for such difficult analytes and exotic analytical approaches in the same way as mentioned previously (Kosman et al. 2021). The main study covered all the important aspects necessary to characterize the original drug pharmacokinetics (SCEEMP 2012).

The dAVT kinetics curves in blood plasma after administering the tested drug at three different doses (Fig. 2) were similar and typical for intravenously administrated drug forms. The maximum plasma concentration of the active ingredient for all the doses was found at the first time point after administration. Further, a gradual decrease in the dAVT plasma concentration was observed. After 24 hours of the experiment, the dAVT content in the rats' blood plasma was less than 0.5% of the maximum detected concentration; in rabbits' plasma, analyte was detected only for the maximum tested dose (the content was less than 0.7% of the maximum detected concentration).

Table 1. Validation Parameters of Methods to Quantify 1-deamino-arginine-vasotocin in Biomaterial of Laboratory Animals

Parameter	Value			
	Blood plasma	Liver	Kidney	Regulated values (FDA 2018, EMA 2011)
Calibration range, µg/ml for plasma or µg/g for tissues	0.008–2.0	0.034–7.5	0.034–8.40	no
Regression equation*	$Y=-0.2281 \cdot X+0.3674$	$Y=0.2128 \cdot X+1.40262$	$Y=1.3171 \cdot X+3,1715$	no
Correlation coefficient, r	0.9986	0.9987	0.9999	0.9–0.99
LLOQ**, µg/ml for plasma or µg/g for tissues	0.0016	0.034	0.034	No requirements
Accuracy, %				
ULOQ**	8.6	6.4	2.4	No more than 15% for
Middle QC	1.8	2.7	1.8	ULOQ, middle and low
Low QC	13.5	3.7	8.9	QC, no more than 20% for
LLOQ	13.7	7.4	5.8	LLOQ
Intra-day//inter-day precision, %				
ULOQ	10.0//13.5	8.2//10.2	10.4//12.5	No more than 15% for
Middle QC	7.4//10.2	6.7//9.8	11.4//9.6	ULOQ, middle and low
Low QC	10.3//12.8	5.3//8.7	8.9//10.8	QC, no more than 20% for
LLOQ	12.8//16.4	10.2//14.3	5.9//11.9	LLOQ

Note: * – Y – change in sodium concentration (Δ), X – logarithm of 1-deamino-arginine-vasotocin concentration (lg C, µg/ml or µg/g); ** – ULOQ – upper limit of quantification, middle QC and low QC – quality control, LLOQ – lower limit of quantification; for blood plasma – 1.88, 0.94, 0.038 and 0.008 µg/ml, for rat liver – 7.5, 2.5, 0.06 and 0.03 µg/g, for rat kidney – 8.4, 4.2, 0.06 and 0.03 µg/g.

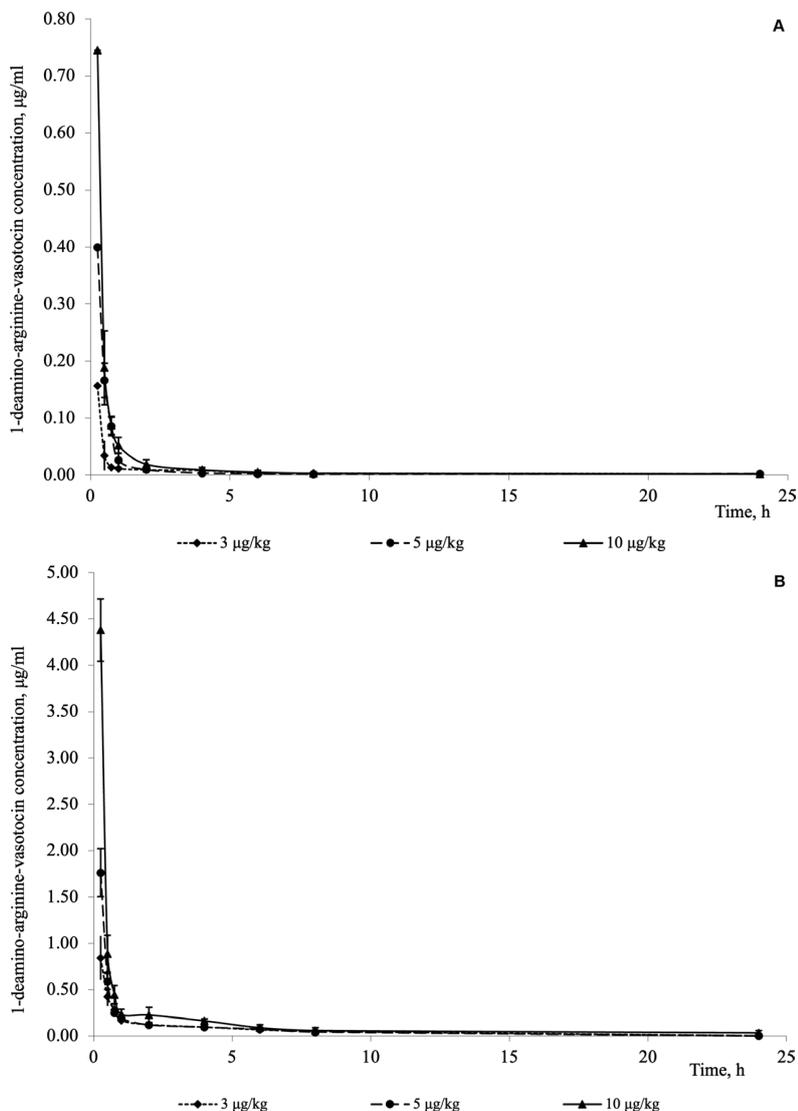


Figure 2. Concentration-time curve of 1-deamino-arginine-vasotocin after single intravenous administration of the tested drug to rats (A) and rabbits (B) (n=5 for rats at a dose of 5 µg/kg, n=6 for all other cases, $(\bar{X} \pm S\bar{X})$ in three doses.

Pharmacokinetic parameters of the tested drug after administration to rats and rabbits are presented in Table 2.

The maximum plasma concentration (C_{max}) values had statistically significant differences ($p < 0.05$) depending on a dose of the administered drug. The average value of this parameter increased about 1.85–2.5 times for the rats and 2–2.5 times for the rabbits with increasing the dose. The calculated parameter C_0 characterized plasma drug concentration immediately after an intravenous injection was higher than the real measured concentration at the first time point (C_{max}) – 3–5 times for the rats and 2–6 times for the rabbits. The main parameter characterizing a degree of bioavailability of the drug, AUC_{0-24} , also had statistically significant differences ($p < 0.05$) between the values for all the studied doses for the two studied animal species. A linear increase in AUC_{0-24} values for rats and rabbits was observed (Fig. 3).

Half-life period values ($T_{1/2}$) were about 6.5–8.5 hours for rats and about 4–6 hours for rabbits. This

parameter was characterized by sufficiently high variability, and the differences for the groups receiving different doses of the drug were statistically insignificant ($p > 0.05$). Total clearance (Cl_T) reflecting pharmacological release volume per time unit for rats was from 5.1 to 17.7 ml/kg/h, for rabbits it ranged from 1.4 to 2.2 ml/kg/h, and had no statistical differences ($p > 0.05$) for the tested doses.

The maximum concentration of dAVT in animals blood plasma was observed about 0.5–1 hour after intramuscular administration of the drug and then a rapid decrease in its concentration was observed, and by the 8th hour of the experiment for rabbits, the concentration of dAVT was about 14% of the maximum (Fig. 4). The rate of dAVT removal from blood plasma was rather high – $T_{1/2}$ was 10.6 ± 2.0 h for rats and 9.2 ± 2.7 for rabbits (Table 2). The relative bioavailability of dAVT after intramuscular administration and after intravenous administration was 32.4% for rats and 40.1% for rabbits.

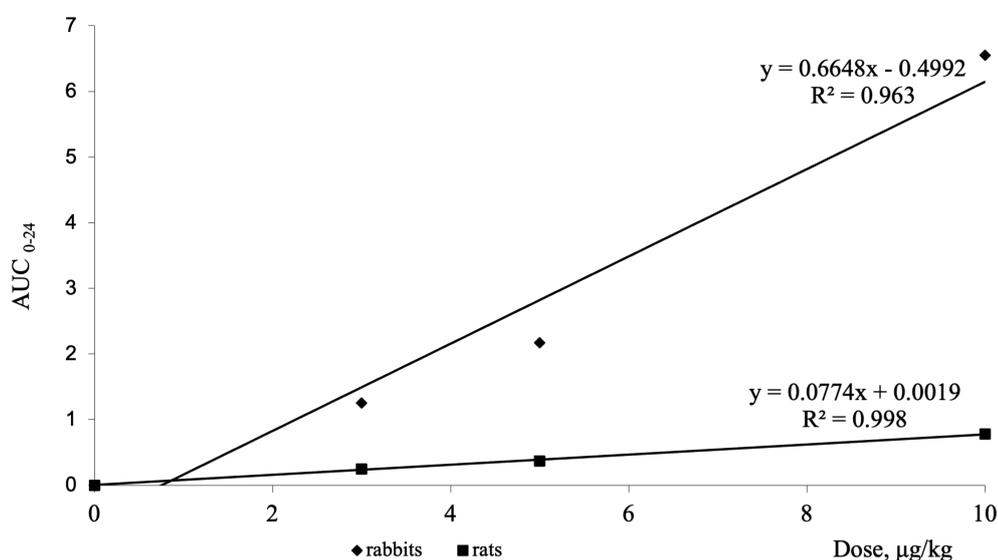


Figure 3. Correlation between AUC_{0-24} values and administrated doses of the tested drug for rats and rabbits.

Table 2. Pharmacokinetic Parameters of 1-deamino-arginine-vasotocin in Blood Plasma of Rats and Rabbits

Dose, µg/kg	Parameter ($\bar{X} \pm \bar{Sx}$, n=5 for single intravenous administration to rats at a dose of 5 µg/kg, n=6 for all other cases)						
	C_0 , µg/ml	C_{max} , µg/ml	T_{max} , h	AUC_{0-24} , h × µg/ml	MRT, h	$T_{1/2}$, h	Cl_T , ml/kg/h
Rats, intravenous, single administration							
3	0.82±0.17	0.16±0.03	–	0.25±0.02	–	8.4±1.5	17.7±1.4
5	1.12±0.37	0.40±0.03	–	0.37±0.05	–	6.6±2.6	13.8±1.3
10	3.13±0.47	0.74±0.06	–	0.78±0.08	–	6.5±1.3	13.7±2.1
Rats, intravenous, repeated administration							
5	0.45±0.08	0.33±0.04	–	0.33±0.04	–	9.0±2.6	15.5±2.1
Rats, intramuscular, single administration							
5	–	0.05±0.00	1.0±0.0	0.12±0.01	8.5±0.9	10.6±2.0	–
Rabbits, intravenous, single administration							
3	1.79±0.59	0.84±0.24	–	1.25±0.23	–	5.3±1.4	1.9±0.2
5	6.89±1.81	1.76±0.23	–	2.17±0.27	–	4.1±0.3	2.2±0.3
10	27.84±6.05	4.38±0.34	–	6.55±0.49	–	5.8±2.5	1.4±0.1
Rabbits, intramuscular, single administration							
5	–	0.44±0.03	0.63±0.06	0.87±0.06	12.9±3.8	9.2±2.7	–

Note: C_0 – concentration immediately after an intravenous injection of the drug, C_{max} – maximum concentration, T_{max} – time to reach maximum concentration, AUC_{0-24} – area under the concentration-time curve, MRT – mean retention time, $T_{1/2}$ – half-life time, Cl_T – clearance, – – not determined for this type of administration.

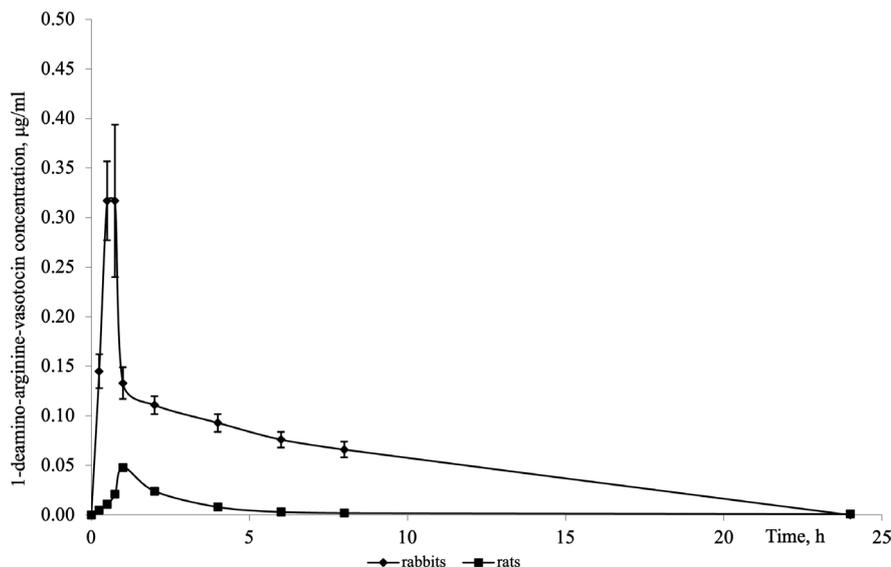


Figure 4. 1-deamino-arginine-vasotocin concentration-time curve after single intramuscular administration of the substance to rats and rabbits at a dose of 5 µg/kg (n = 6, $(\bar{X} \pm S\bar{x})$).

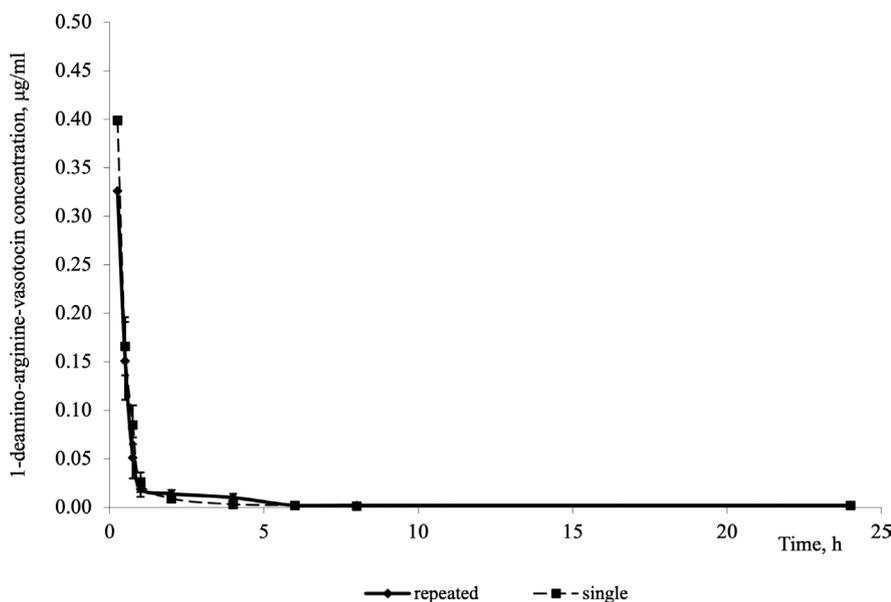


Figure 5. 1-deamino-arginine-vasotocin concentration-time curve after single and repeated intravenous administration of the tested drug to rats at a dose of 5 µg/kg (n=5 for single administration, n=6 for repeated administration, $(\bar{X} \pm S\bar{x})$).

The main goal of pharmacokinetic research with repeated drug administration is to clarify its ability to accumulate in the body. Since the linearity of pharmacokinetics was determined in the experiment with a single administration, one dose level – 5 mg/kg – was used for repeated administration, with the drug administered once a day for 3 days.

On the one hand, the sodium level in the test animals during the experiment may depend on the influence of various physiological factors. At the same time, the initial values of the sodium level, measured at the "zero" points before the first and last drug administration, were $136.0 \pm 35.7 \mu\text{mol/L}$ and $137.2 \pm 27.3 \mu\text{mol/L}$, respectively, had no statistically significant differences and did not go beyond the limits of intra-laboratory physiological norms

for this species of animals. The values of parameter Δ (the proportion of a change in sodium concentration taking into account the levels before and after the drug administration) used for further calculations of the dAVT content were similar at the corresponding time points after multiple and single administrations. Based on the biomarker content change, the curves of dAVT kinetic in blood plasma after repeated and single tested drug administrations at a dose of 5 µg/kg were similar (Fig. 5); active ingredient concentrations for all the time points and the values of the main pharmacokinetic parameters – C_{max} , AUC_{0-24} , $T_{1/2}$, Cl_T (Table 2) had no statistically significant differences ($p > 0.05$) after repeated and single administrations. Thus, the absence of active substance cumulation after tested drug intravenous administration was suggested.

Tissue availability of the drug is an important step in pharmacokinetic studies. The main result of the distribution processes is the drug transport to the area of action, where it interacts with structures that determine the effect of the drug.

The intensity of penetration of the pharmacological agent into peripheral tissues should be characterized by tissue availability (f_t), defined as the ratio of the tissue AUC value (AUC_T) to the corresponding plasma AUC value, as well as the value of the half-life and mean retention time ($T_{1/2,T}$ and MRT_T) (SCEEMP 2012). The main parameters characterizing dAVT penetration into liver, kidneys and spleen are presented in Table 3. The dAVT content was lower than LLOQ in the muscle and omentum samples obtained during the experiment.

Table 3. Main Parameters of 1-deamino-arginine-vasotocin Penetration Into Rats Liver, Kidneys and Spleen After a Single Intravenous Administration of the Tested Drug at a Dose of 5 $\mu\text{g}/\text{kg}$

Tissue	AUC_{0-24} , $\text{h} \times \mu\text{g}/\text{ml}$	MRT_T , h	$T_{1/2,T}$, h
Liver	0.72 ± 0.05	4.9 ± 0.2	2.6 ± 0.2
Kidney	0.55 ± 0.12	11.4 ± 3.9	7.3 ± 2.9
Spleen	1.64 ± 0.16	2.6 ± 0.2	1.50 ± 0.13

Note: AUC_{0-24} – area under the concentration-time curve, MRT_T – tissue mean retention time, $T_{1/2,T}$ – tissue half-life time.

The dAVT tissue availability values (f_t) for the liver was 1.98, for the kidneys – 1.5, for the spleen – 4.47 (Fig. 6). The high concentration of the active substance was observed in the spleen, liver and kidneys. These data suggest intensive distribution of dAVT into highly vascularized tissues (spleen), intensive metabolism and subsequent excretion in the liver and kidneys, and low distribution into moderately and weakly vascularized tissues (muscles, omentum).

No dAVT was found in feces samples; its maximum concentration in urine was observed in the interval of 2–4 hour after single administration of the tested drug at a dose of 5 $\mu\text{g}/\text{kg}$. In total, less than 10% dAVT was removed from the rat body with urine over 24 hours of the experiment.

Conclusion

The pharmacokinetics parameters of nonapeptide drug dAVT in the form of injection solution were evaluated, including absorption after single administration at three

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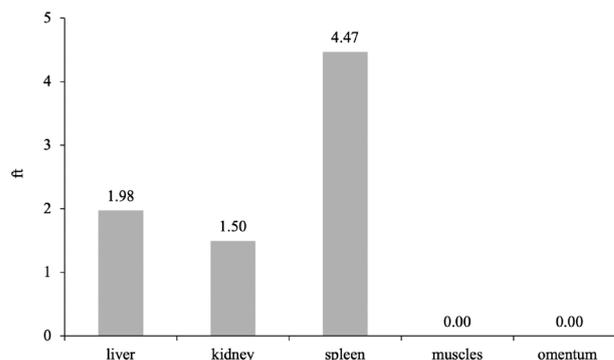


Figure 6. Tissue availability (f_t) of 1-deamino-arginine-vasotocin after a single intravenous administration of the tested drug at a dose of 5 $\mu\text{g}/\text{kg}$.

doses in two types of animals, as well as linearity of pharmacokinetics and relative bioavailability, distribution to tissues, excretion with two types of excretes, and evaluation of pharmacokinetics with repeated administration at one dose.

The pharmacokinetics of the drug was found to be linear after single administration of dAVT drug in the dose range of 3–10 $\mu\text{g}/\text{kg}$ for rats and rabbits. The relative bioavailability of dAVT after intramuscular and intravenous administrations was more than 30%.

The active substance was intensively distributed into highly vascularized tissues (spleen), tissues providing metabolism and excretion (liver and kidneys), and hardly reached moderately and weakly vascularized tissues (muscles, omentum). Less than 10% of dAVT was excreted with urine; it was not determined in feces; and repeated administration did not lead to its cumulation.

The study included all the main areas necessary to characterize the original drug pharmacokinetics.

Conflict of interest

The authors have declared that no competing interests exist.

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