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Avdeeva N.V.¹,
Kulikov A.L.²,
Pokrovskii M.V.³,
Avtina T.V.⁴

PHARMACOKINETIC STUDIES OF NEW ANTIPARKINSONIAN DRUG RAPITALAM

- 1) PhD of Medical Sciences, Assistant of Department of internal diseases №2 of Kursk State Medical University 3, Karl Marx St., Kursk, 305041, Russia, e-mail: 7400468@mail.ru
- 2) Postgraduate student of the Department of Pharmacology of Medical institute of Belgorod State University 85, Pobedy St., Belgorod, 308015, Russia, e-mail: kulikov@bsu.edu.ru
- 3) Doctor of Medicine, Prof.; Head of the Department of Pharmacology of Belgorod State University 85, Pobedy St., Belgorod, 308015, Russia, e-mail: pokrovskii@bsu.edu.ru
- 4) PhD in Pharmaceutical Sciences, Associate Professor, Department of Pharmacology of Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia, e-mail: avtina_t_@bsu.edu.ru

Abstract

Parkinson's disease is the most common neurodegenerative disorder after Alzheimer's disease. The aim of this study was to investigate the pharmacokinetic parameters of the mGluR4 receptor blocker Rapitalam on rabbits. There was developed the method of the quantitative determination of Rapitalam in the blood plasma of rabbits using high performance liquid chromatography with tandem mass spectrometric detection. The study was performed on 12 rabbits (males, weighing between 3,300 to 3,500 g). In intragastric dosing of the substance was administered using a gastric tube in the form of suspension in water 0.9 mg/ml, 9 mg/ml, and 90 mg/ml at a dose of 0.3 mg/kg, 3 mg/kg and 30 mg/kg. After the administration of the substance blood was sampled through a catheter in a volume of 0.5 ml in polypropylene tubes containing 20 µl of 5% EDTA before and 10, 15, 30, 60, 120, 240, 480, 1440 minutes after administration. The mean absorption time (MAT) of Rapitalam was 268.1 minutes or 4.5 hours. The half-life is longtime and it was 176.4 minutes (2.9 hours) for intravenous and 362.2 minutes (6.0 hours) for intragastric administration. The absolute bioavailability of the intragastric dosing was 26.8%. The main pharmacokinetic parameters of the substance was established on rabbits that allow you to optimize the future use of it's as a potential drug for the treatment of Parkinson's disease.

Key words: Rapitalam, Parkinson's disease, the blood plasma of rabbits, high performance liquid chromatography, pharmacokinetic, metabotropic glutamate receptors.

Introduction

Parkinson's disease belongs to a group of neurodegenerative diseases of the brain. The main symptoms of Parkinson's are the loss of coordination, constraint and slowness when walking, tremor (shaking) of hands, feet, chin. Most often Parkinson's disease affects the elderly [1]. The pathogenesis of the disease is the insufficient synthesis of dopamine in the substantia nigra and striatum. Dopamine

replacement therapy is currently the primary approach in the treatment of Parkinson's disease. However, levodopa does not slow down the continued degeneration of dopaminergic neurons, the functional activity of which causes the conversion of levodopa into dopamine by action of DOPA decarboxylase [6]. Also drugs of levodopa group have a large number of side effects, and over time, the dosage must be increased, otherwise they lose

effectiveness. Therefore, the attending physician of a patient suffering from Parkinson's disease has two main tasks: to suspend the death of ganglion containing dopamine, and reduce the symptom load of disease [1]. Progress in the understanding of the anatomy and function of basal ganglia has provided an opportunity for the development of new drugs for the treatment and slowing the progression of Parkinson's disease [3]. Although they are trying to cure Parkinson's disease, the experts can only partially eliminate the symptoms but not the cause itself. Today, all the efforts of scientists aimed at finding drugs that will not only soften the symptoms of the disease, but also stopped the degenerative processes responsible for its progression. So glutamate receptors have been proposed as promising therapeutic targets, since the effects on them allow you to change both normal and pathological neurotransmission characteristic of the Parkinsonian brain.

The above data indicate the feasibility of preclinical study of Rapitalam, mGluR4 receptor modulator, to create on its basis drugs that have anti-Parkinsonian effect [6].

The aim of this study was to investigate the pharmacokinetic parameters of the mGluR4 receptor blocker Rapitalam on rabbits.

Materials and methods

There was studied a Rapitalam substance, white powder. Rabbits are common animals used in studies of pharmacokinetics in accordance with the "Manual on experimental (preclinical) study of new pharmacological substances" [6]. Moreover, these animals are economically beneficial, because in the preliminary catheterization of the animals, to take samples of blood from each animal at all time points. The study was performed on 12 rabbits (males, weighing between 3,300 to 3,500 g). During the period of adaptation of animals, which is lasted 7 days, there was carried out daily inspection of their external condition. For the study there were selected rabbits with no signs of abnormalities in appearance. All animals were kept in separate rooms. Rabbits were kept by two in steel lattice coops. After a period of adaptation the animals were catheterized in the right ear vein and kept individually. The basic rules of keep and care consistent with the standards given in the manual • Guide for the care and use of laboratory animals. The National Academy press. –Washington, D.C. 2011 [9] and regulations approved by all-Union

State Standard 31886-2012 "Principles of good laboratory practice". 12 hours before the start of the experiment, catheterized animals were deprived of feed with free access to water. The test substance was administered on the third day after catheterization. Intravenous dosing the test substance was administered bolus of 6 rabbits in the ear vein in the form of a solution 9 mg/ml in propylene glycol at a dose of 3 mg/kg. In intragastric dosing of the substance was administered using a gastric tube in the form of suspension in water 0.9 mg/ml, 9 mg/ml, and 90 mg/ml at a dose of 0.3 mg/kg, 3 mg/kg and 30 mg/kg. After the administration of the substance blood was sampled through a catheter in a volume of 0.5 ml in polypropylene tubes containing 20 µl of 5% EDTA before and 10, 15, 30, 60, 120, 240, 480, 1440 minutes after administration. Blood plasma was separated by centrifugation at 5600 g for 10 min and stored until analysis at -70 C.

Sample preparation blood plasma samples was performed by protein precipitation with methanol. To do this, into a test tube type "Eppendorf" with a capacity of 1.5 ml there were added 0.2 µl of plasma and 50 µl of internal standard and mixed, after there was added 500 µl of MeOH and shaken on a shaker for 15 minutes. Then there was performed the extraction of the analyte into ultrasonic bath for 15 minutes. Then the samples were centrifuged at 13000 rpm and 4 °C temperature for 30 minutes. The supernatant is carefully decanted into vials for chromatography and analyzed.

In quality control solutions and to construct the calibration curve as placebo there was used zero blood plasma of rabbits. Zero samples of rabbits' blood plasma were prepared in addition to calibration standards to confirm the selectivity of the method. The quality control solutions were analyzed during the researches and they found the error between an administered and found quantity of the analyte, compared with margins that served as a validation of a significance of the obtained results.

Rapitalam concentration in the blood plasma of rabbits was determined using the previously developed method of high performance liquid chromatography with tandem mass spectrometric detection which has high sensitivity and selectivity, that allows to determine low concentrations of drug (at the level of ng/ml) in various biological matrices [13]. Brief characteristic of the method is given in table 1.

Table 1.

The conditions of analysis.

<i>Apparatus</i>	
Liquid chromatograph	Thermo Scientific Dionex UltiMate 3000 RS
Detector	Thermo Scientific Velos Pro c HESI.
<i>Chromatographic conditions</i>	
Guard column	Zorbax Eclipse XDB C18 12.5×3.0 mm with a particle size of 5.0 μm
Column	Acclaim™ 120 C18 150×2.1 mm with a particle size of 5.0 μm
Separate mode	Isocratic
Mobile phase	5 mM Ammonium acetate +0.1% Formic acid: MeCN (60:40)
Flow rate	0.3 mL/min
Temperature of samples	5 °C
Temperature of column	40 °C
Volume of injection	1 μL
Retention time of Rapitalam	about 7 min
Retention time of IS	about 4 min
Ionization type	ESI «+»
Mass transformation	Rapitalam: 383,84→367,0; IS: 244,7→130,94.
Temperature of source	300 °C
Voltage across source	3000 V
The remaining parameters are in accordance with an automatic optimization tool.	

Main pharmacokinetic parameters were calculated in accordance with the methodological recommendations for conducting preclinical studies of drugs under the editorship of A. N. Mironov [2]. Due to Microsoft Office Excel 2010 on the basis of the experimentally obtained data there were calculated the pharmacokinetic parameters. Outliers in each time point were identified using a statistical test of Grubbs [4]. This method showed good and accurate results [10, 11, 12]. Arithmetic mean values and the coefficient of variation (CV) were calculated in 6 animals.

The peak areas of the analyte and internal standard 2 were calculated by specialized software Xcalibur 2, then the data were transferred to a Microsoft Office Excel 2010, where we calculated the equation of the calibration curve, statistically evaluated deviation, graphically displayed the results [13]. Rapitalam concentration in the studied samples was calculated in Microsoft Office Excel 2010 from the calibration curve. Outliers in each time point were identified using a statistical test of Grubbs [4]. If any

sample value of Z was greater than the critical value for a given number of dimensions N, this sample was excluded from further calculation of pharmacokinetic parameters. So, for N=6 the critical value of Z is equal to 1.89, so samples with $Z > 1.89$ were considered outliers.

Research results

Into BelSU Clinical and Preclinical Studies Centre there was studied the pharmacokinetic of Rapitalam in the blood plasma of rabbits after a single intravenous administration in dose of 3 mg/kg and intragastric administration in the dose of 30 mg/kg. After analyzing the obtained results, it was identified that after intragastric administration the maximum concentration of Rapitalam in the blood plasma of rabbits is achieved after an average of 480 minutes (4.0 hours) (Fig. 1). The half-life is longtime (362.2 minutes or 6.04 hours). The mean absorption time (MAT) of Rapitalam was 268.1 min (4.47 hours). The absolute bioavailability f_a (%) of Rapitalam after intragastric administration to rabbits was 26.8 %.

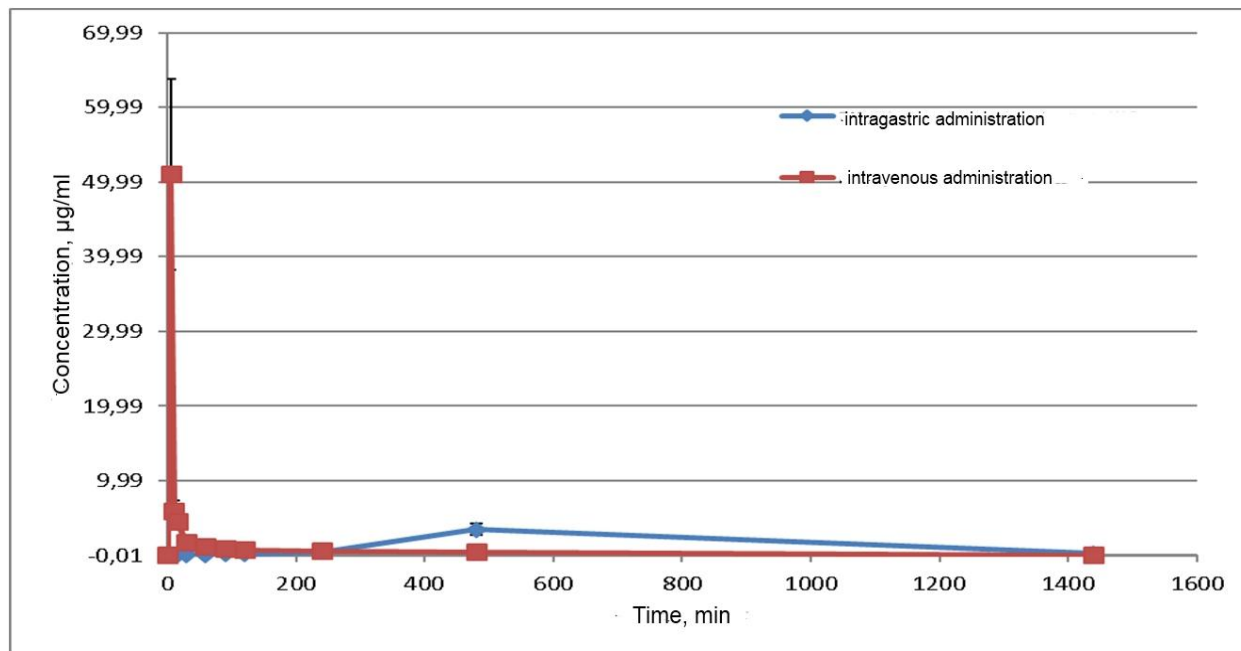


Figure 1. The averaged pharmacokinetic curves of Rapitalam in the blood plasma of rabbits after single administration.

The linearity of the pharmacokinetic of Rapitalam was studied after a single intragastric administration of the drug to rabbits in three increasing dosages of 0.3 mg/kg, 3.0 mg/kg and 30 mg/kg. Based on these data, there was the hypothesis of linearity of the pharmacokinetic of Rapitalam. To

test this hypothesis there was assessed statistically significant departures from zero the intercept $AUC_{(0-1440)}$. The calculation is presented in figure 2. The results showed that the free member is insignificant different from zero and the hypothesis should be considered correct.

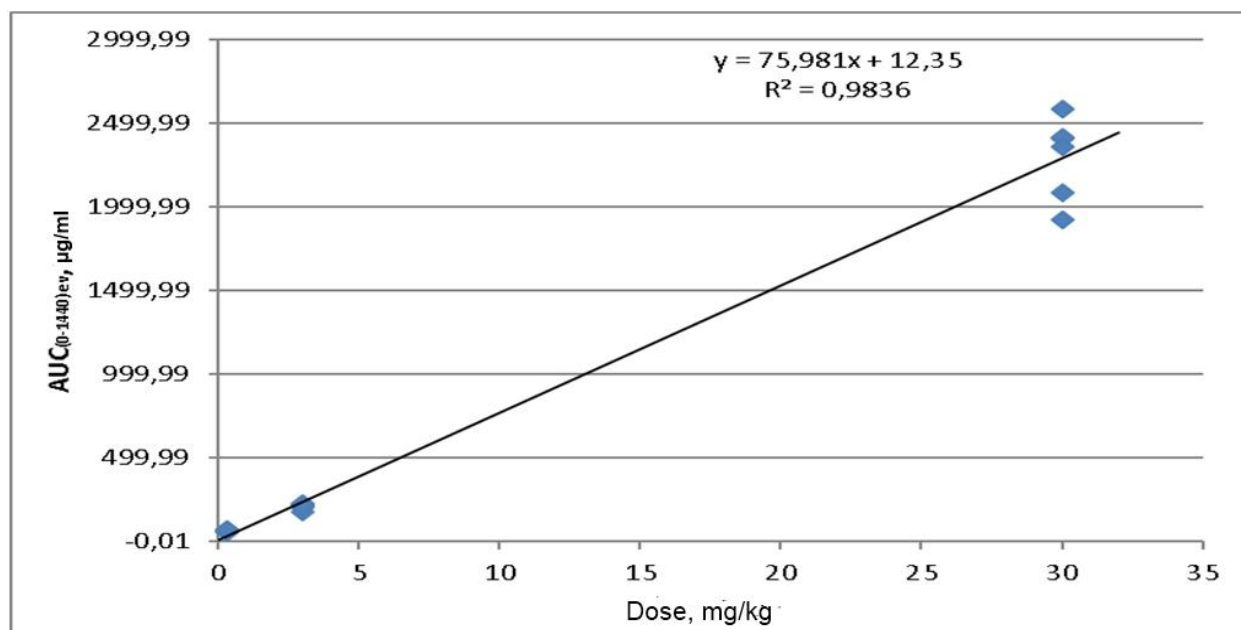


Figure 2. Evaluation of the linear dependence of the $AUC_{(0-1440)ev}$ on dose.

To further evaluate the linearity there were constructed concentration influence curves,

normalized for dose, from time. The results are presented in figure 3.

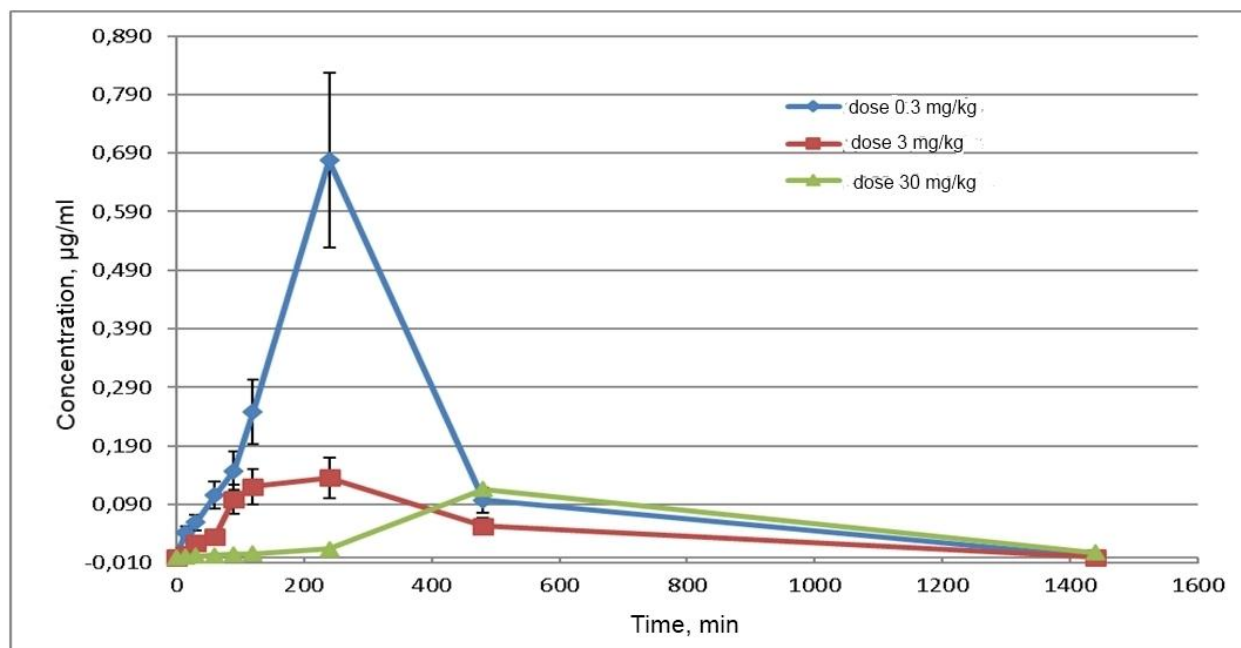


Figure 3. The averaged pharmacokinetic concentration curves of Rapitalam in blood plasma of rabbits (normalized for dose) after a single injection.

From figure 3 it can be seen that the concentrations, normalized by dose, for the different dosages have the same nature and similar value. It is observed excess of maximum concentration for the dose of 0.3 mg/kg over others. This deviation should be a consequence of the higher solubility of a low dose in the fluid of the gastrointestinal tract, at low concentration the effect of solubilization and uniform distribution of Rapitalam throughout the volume. At high concentrations, due to the hydrophobic properties of the test substance, there is observed the effect of agglomeration between the particles, which causes differences in normalized concentrations. It should be noted that the dependence of the $AUC_{(0-1440)_{ev}}$ on dose is linear, and it gives grounds to confirm the linearity of the studied range of Rapitalam in the range from 0 to 30 mg/kg for rabbits.

Conclusions

1. Performed research of the main pharmacokinetic parameters of Rapitalam allowed to develop a method of quantitative determination of this substance in the blood plasma of rabbits.

2. The results obtained in the study of pharmacokinetic of Rapitalam showed that the decrease in its concentration in blood plasma of treated animals is quick in a biexponential manner. The mean absorption time (MAT) of Rapitalam was 268.1 minutes or 4.5 hours. The half-life is longtime and it was 176.4 minutes (2.9 hours) for intravenous and 362.2 minutes (6.0 hours) for intragastric administration. The absolute bioavailability of the intragastric dosing was 26.8%. The maximum

concentration of Rapitalam was observed on average after 4 hours.

3. The study of the linearity of the pharmacokinetic showed that the response/dose in a dosing interval from 0 to 30 µg/kg in rabbits has a linear dependence.

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