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Research Article

In vivo study of pharmacokinetic parameters of a new combination drug based on citicoline and memantine

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Abstract

Introduction: Cognitive impairment (dementia) is one of the most common pathologies with increasing numbers of patients. Most often they are the symptoms of Alzheimer's disease and vascular brain diseases, for which such drugs as memantine and citicoline are used. The development of a combination drug with these active pharmaceutical ingredients can significantly increase the effectiveness of therapy.

Materials and methods: The pharmacokinetics of memantine and citicoline combination drug was evaluated in comparison with the marketed drugs (reference drugs) of these pharmaceutical substances approved for medical use by determining their content in blood plasma of experimental animals after a single oral administration.

Results: Seventy-two hours after the administration of memantine drug, about 5% and 17% of the maximum concentration of memantine released from Akatinol Memantine and the developed combination drug were found in blood plasma, respectively. By the 120th hour after the beginning of the experiment, no memantine was detected in blood plasma of any animal. By the 24th hour after the beginning of the experiment, about 46% and 50% of the maximum concentration of citicoline released from the developed combination drug and the Ceraxon drug were found in blood plasma of the rabbits, respectively.

Discussion: It was detected that the amounts of released memantine and citicoline from the developed combination drug exceeded the amounts of the appropriate pharmaceutical substances released from the reference drugs. The bioavailability of these substances from the developed combination drug was higher than from the marketed mono formulations used as reference drugs.

Conclusion: Based on the obtained results of memantine and citicoline concentrations in bioassays, the main pharmacokinetic parameters of the studied preparations were calculated, the results of which showed the superiority of the developed combination drug over the reference drugs.

Keywords

citicoline, memantine, pharmacokinetics.

Introduction

According to the World Health Organization, over 35 million people in the world suffer from severe forms of cognitive impairment. At the same time, the annual growth rate is more than 7 million new cases of dementia, which allows forecasting a double increase in the number of patients with their various forms by 2030 and a triple increase – by 2050 (World Health Organization 2012). From the etiology point of view, cognitive impairments are a heterogeneous group of disorders, but most of them are symptoms of Alzheimer's disease and vascular brain diseases (Kurushina and Vorobieva 2019). Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the cause of dementia in 60% of all cases (Jellinger 2014).

Citicoline is an endogenous mononucleotide, which is normally present in all cells of the human body. Due to a molecule element (choline), citicoline is involved in the synthesis of structural phospholipids of cell membranes, being an intermediate compound in the formation of phosphatidylcholine in the phospholipid synthesis (Trofimova and Preobrazhenskaya 2015). The therapeutic efficacy of citicoline in AD has been shown in a number of controlled clinical trials, which have showed the positive effects of citicoline on the long-term course of dementia and drug safety (Dorovskikh 2015).

In turn, memantine is an reversible non-competitive antagonist of N-methyl-D-aspartate receptors, recommended for use in the stages of moderate to severe dementia that develops in AD (Kicherova and Reikhert 2018; Mendelevich 2018).

The combined use of citicoline and memantine had a synergistic effect and slowed down AD progression with mixed dementia in patients (Gareri et al. 2018), which makes drug development containing both of these active pharmaceutical ingredients promising.

The study of pharmacokinetics is an integral part of preclinical studies of the pharmacological agents required for registration of a drug product and is subject to the requirements of regulatory documents (Guidelines for Conducting Drug Preclinical Trials 2012). The purpose of the present research was to study the pharmacokinetics of the developed drug in the form of tablets, containing a combination of memantine and citicoline, in comparison with the registered drugs; Akatinol Memantine (INN memantine) coated tablets, 10 mg (Merz Pharma GmbH & Co. KGaA (Germany)) and Ceraxon (INN citicoline) 1000 mg, oral solution, 100 mg/ml (Ferrer International, S.A., Spain).

Materials and methods

Experimental animals

The research was carried out on sexually mature male New Zealand rabbits, with a single oral administration, similar to the clinical practice. The drugs were administered to the animals one tablet at a time, without crushing, but using a tablet dispenser.

To exclude the influence of the researcher's preferences on the experimental group formation, the selection of animals was carried out using a modified block randomization procedure (Guidelines for Conducting Drug Preclinical Trials 2012). In that case, the rabbits to be used in the study were randomly placed in cages for randomization (the number of cages for randomization is multiples of the number of the experimental groups). Then using a random number generator (STATISTICA 10.0, StatSoft, USA), a data list was obtained, containing the numbers of the animal cells and the corresponding group numbers where the animals were placed later (Altman 1999; Bland 2000). The rabbits were divided into 4 groups of 6 animals each. For the experimental groups, the animals without signs of deviations in appearance were selected, so that the individual weight did not deviate from the average value of the groups by more than 20%.

The animals were kept in standard conditions according to the sanitary and epidemiological requirements of SP 2.2.1.3218-14 *Sanitary and Epidemiological Requirements for the Design*, *Equipment and Maintenance of Experimental Biological Clinics* (*Vivariums*) (approved by Ordinance № 51 of the Chief Public Sanitary Inspector of the Russian Federation of August 29, 2014,) and Directive 2010/63/EU of the European Parliament and the Council of the European Union from 22 September 2010 on the protection of animals used for scientific purposes.

Each rabbit was catheterized with a 22G intravenous catheter (KD Medical GmbH Hospital Products, Germany) in the marginal ear vein, through which blood samples were taken in a volume of 2.0 ml per exposure point. This pharmacokinetic study was discussed during the meeting of the Bioethical Commission of JSC "HOME OF PHARMACY" and approved for being conducted (Final report of the Bioethical Commission No. 2.66/18 of 22.10.2018).

Drug products under study

A developed combination drug (Citicoline+Memantine), in form of modified release film-coated tablets, 500 mg+10 mg, is an object of the study.

Approved drug products were used as reference drugs: Ceraxon (INN citicoline) 1000 mg, oral solution, 100 mg/ml (Ferrer International, S.A., Spain) and Akatinol Memantin (INN memantine) film coated tablets, 10 mg (Merz Pharma GmbH & Co. KGaA (Germany)).

Design of experiment

With a single injection at 0 o'clock, the biomaterial was sampled before the administration of the drugs. Then the studied drugs were injected, followed by sampling biomaterial at the appropriate time points of 0, 30 minutes, 1, 2, 4, 6, 8, 24, 48, 72 and 120 hours. All the drugs were administered to the animals once, orally. The drug Ceraxon

was administered in an amount of 5 ml (which is equal to a dose of 500 mg). Akatinol Memantine was administered in the amount of 1 tablet, without crushing. The developed combination drug was administered in the amount of 1 tablet, without crushing.

Determination of concentration

The blood was taken into heparinized tubes, after which it was centrifuged for 15 min at 3000 rpm. After centrifugation, the plasma was carefully collected into 1.5-ml plastic tubes (Eppendorf type) and frozen at -20 °C. Before starting a quantitative determination of the active ingredient, the samples were unfrozen.

Citicoline and Memantine concentrations in the biological samples were determined by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection, validated in the concentration range from 30 to 11500 ng/ml of citicoline and from 50 to 83.500 ng/ml of memantine (in the form of o-phthalaldehyde (OPA) derivatives) (Sandhya et al. 2014; Dousa et al. 2016; El-Hamamsy et al. 2017; Abbaszadeh et al. 2018). The procedures are validated in terms of: selectivity, Lower Level of Quantification (LLOQ), calibration range, accuracy, and precision. Satisfactory results were obtained for all the validation parameters, which indicates a possibility of further use of the developed procedures to study the pharmacokinetics of the drug on rabbits.

In accordance with the recommendations, citicoline concentration, corresponding to the smallest point of the calibration curve, was accepted as LLOQ.

Validation parameters of citicoline and memantine determination in rabbit blood plasma are presented in Tables 1, 2.

Table 1. Validation Parameters of Citicoline Determination in Rabbit Blood Plasma

Validation parameters	Value
Calibration range	30-11500 ng/ml
Regressional relationship*	Y=15.514·X+176.29
Correlation coefficient r	0.9995
LLOQ	30 ng/ml
Accuracy, %	
11500 ng/ml	1.9
5000 ng/ml	4.9
75 ng/ml	4.0
30 ng/ml	7.4
Precision, %	
11500 ng/ml	1.2
5000 ng/ml	1.4
75 ng/ml	6.4
30 ng/ml	8.0

Note: * Y – citicoline peak area, X – citicoline concentration, ng/ml.

Statistical analysis

Registration and processing of chromatograms were made by using LABSOLUTIONS LCSOLUTION software, Version 1.25 (Shimadzu, Japan).

At the zero point (before drug administration), the study of blood plasma samples detected no analytes.

Table 2. Validation Parameters of Memantine Determination in Rabbit Blood Plasma

Validation parameters	Value
Calibration range	50–83500 ng/ml
Regressional relationship*	Y=28.278·X+48.626
Correlation coefficient r	0.9990
LLOQ	50 ng/ml
Accuracy, %	
83500 ng/ml	0.1
60000 ng/ml	0.4
100 ng/ml	11.1
50 ng/ml	7.2
Precision, %	
83500 ng/ml	4.2
60000 ng/ml	1.0
100 ng/ml	9.5
50 ng/ml	13.0

Note: * Y – memantine peak area, X – memantine concentration, ng/ml.

The pharmacokinetic parameters (maximum concentration C_{max}, time to reach maximum observed concentration T_{max}, area under curve AUC, mean retention time MRT, half-life $T_{1/2}$ and rate of absorption C_{max}/AUC_t) were calculated by a model-independent method of statistical moments. For the statistical estimation of the differences between the pharmacokinetic parameters, a two-tailed t-test for the averages was used (the assessment was carried out at a 95% confidence level) in EXCEL 2010 (Microsoft, USA). Such an approach is acceptable in case of normal distribution and independence of the analyzed samples.

The pharmacokinetic parameters were calculated by the model-independent method of statistical moments, using the pKsolver program. The tables present the arithmetic mean values ($\bar{X}$), their corresponding standard deviations (SD), and standard errors of the mean ($S\bar{x}$).

The pharmacokinetic parameters were calculated by a model-independent method – the method of statistical moments, since with the compartment approach, various pharmacokinetic parameters can be obtained, which can significantly affect the study results.

Calculation of relative bioavailability was performed according to the following formula (1):

$$f = \frac{\left(AUC_{0-\infty}\right) \text{ tested drug product}}{\left(AUC_{0-\infty}\right) \text{ reference drug product}} \quad (1)$$

Standard deviation of the obtained value was assessed according to the following formula (2):

$$\text{SD} = \sqrt{SD \text{ reference drug product}^{\,2} + SD \text{ tested drug product}^{\,2}} \quad (2)$$

Calculation of relative bioavailability was performed according to the above formula (3) using average [AUC]_(0-∞) values from Tables 7, 8 (as regards to citicoline):

$$f(\text{ relative bioavailability }) = \frac{5095.99}{3995.64} * 100\% = 127,7\% \quad (3)$$

Since when assessing relative bioavailability, the AUC value obtained for the reference drug is accepted as 100%, the relative bioavailability of the tested drug can be either less or more than 100%, because it can be either less (worse) or more (better) in comparison with the reference drug.

Results

The dynamics of changes in the concentrations of memantine in the blood plasma of rabbits after a single administration of the drugs is presented in Table 3. The averaged pharmacokinetic curves in linear and semi-logarithmic coordinates are shown in Figures 1, 2.

Table 3. The Memantine Content in Blood Plasma of Rabbits After a Single Oral Administration of the Developed Combination Drug and Akatinol Memantine, ng/ml

Developed combination drug				Akatinol Memantine			
Time, h	Average, ng/ml	SD	Sx	Time, h	Average, ng/ml	SD	Sx
0.5	0.00	0.00	0.00	0.5	0.00	0.00	0.00
1	66.33	15.87	6.48	1	107.00	14.03	5.73
2	97.00	29.43	12.01	2	136.67	34.06	13.91
4	413.00	290.11	118.44	4	191.17	98.93	40.39
6	496.67	261.60	106.80	6	250.33	133.15	54.36
8	427.50	260.97	106.54	8	312.83	187.61	76.59
24	176.17	36.82	15.03	24	194.83	85.87	35.06
48	143.17	49.64	20.27	48	126.67	28.74	11.73
72	43.67	34.23	13.97	72	102.17	35.36	14.44
120	0.00	0.00	0.00	120	69.67	9.46	3.86

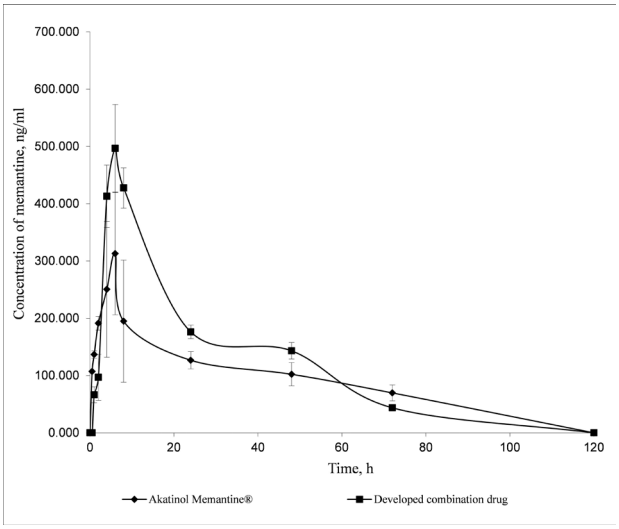

Figure 1. The "concentration-time" curve of memantine after a single oral administration of the developed combination drug and Akatinol Memantine (n=6, $\bar{X} \pm S\bar{x}$).

The detection kinetics of memantine in blood plasma after administration of the developed combination drug and Akatinol Memantine was similar (Figs 1, 2). The shape of the obtained kinetic curves is characteristic of those for dosage forms used orally.

The maximum concentration of memantine in blood plasma during administration of the developed combination drug was observed in the interval of 4–8 hours, with the administration of Akatinol Memantine – in the interval of 6–8 hours. Further, a gradual decrease in the concentration of memantine in blood plasma was observed. From the literature, it is known that the value of the T_{max} parameter after a single oral dose by healthy volunteers was 3–8 hours (Gomolin et al. 2010).

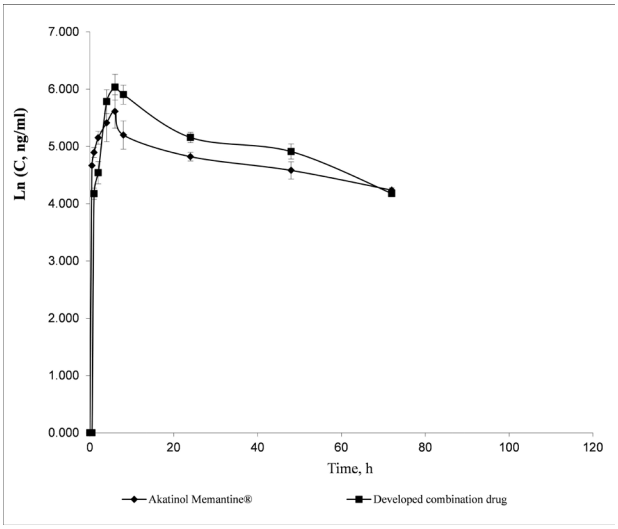

Figure 2. The "concentration-time" curve of memantine after a single oral administration of the developed combination drug and Akatinol Memantine in semi-logarithmic coordinates (n=6, $\bar{X} \pm S\bar{x}$).

Seventy-two hours after the administration of the drugs, about 5% and 17% of the maximum concentration of memantine released from Akatinol Memantine and the developed combination drug were found in blood plasma, respectively. One hundred twenty hours after the beginning of the experiment, no memantine was detected in blood plasma of all the animals.

As it can be seen from the data presented in Figures 1, 2, the obtained kinetic curves characterizing the content of memantine in blood plasma of the rabbits after administration of the developed combination drug and Akatinol Memantine show that the profile of the kinetic curve of the memantine release from the developed drug is quite similar to that from the reference drug Akatinol Memantine.

The dynamics of changes in the concentrations of citicoline in blood plasma of the rabbits after a single administration of the drugs is presented in Table 4. The averaged pharmacokinetic curves in linear and semi-logarithmic coordinates are shown in Figures 3, 4.

The detection kinetics of citicoline in blood plasma after administering the developed combination drug and

Table 4. The Citicoline Content in Blood Plasma of Rabbits After a Single Oral Administration of the Developed Combination Drug and Ceraxon, ng/ml

Developed combination drug				Ceraxon			
Time, h	Average, ng/ml	SD	Sx	Time, h	Average, ng/ml	SD	Sx
0.5	91.33	54.68	22.32	0.5	32.50	1.52	0.62
1	131.83	57.45	23.46	1	41.00	6.13	2.50
2	157.50	70.85	28.92	2	58.00	15.26	6.23
4	166.67	84.93	34.67	4	72.67	25.07	10.23
6	155.17	83.87	34.24	6	67.00	22.65	9.24
8	128.50	66.90	27.31	8	63.67	23.43	9.57
24	76.50	31.42	12.83	24	43.67	7.17	2.93
48	0.00	0.00	0.00	48	0.00	0.00	0.00
72	0.00	0.00	0.00	72	0.00	0.00	0.00
120	0.00	0.00	0.00	120	0.00	0.00	0.00

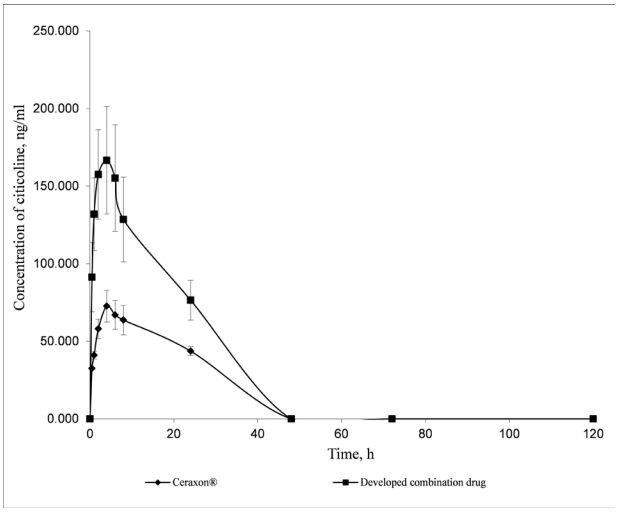

Figure 3. The "concentration-time" curve of citicoline after a single oral administration of the developed combination drug and Ceraxon, rabbits (n=6, $\bar{X} \pm S\bar{x}$).

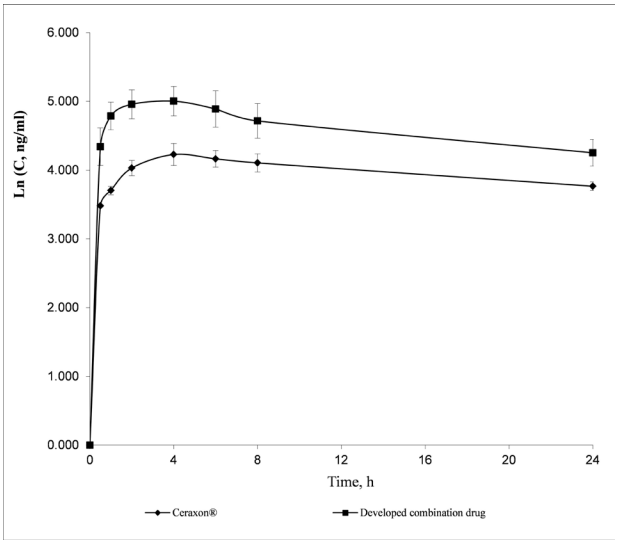

Figure 4. The "concentration-time" curve of citicoline after a single oral administration of the developed combination drug and Ceraxon, rabbits, in semi-logarithmic coordinates (n=6, $\bar{X} \pm S\bar{x}$).

Ceraxon was similar and had the same shape of the curve – a "breaking wave" shape. The maximum concentration of citicoline in blood plasma with the introduction of the developed combination drug was observed in the interval of 1–6 hours, and with the introduction of Ceraxon – in the interval of 2–6 hours. Further, a gradual decrease in the concentration of citicoline in blood plasma was observed. By the 24th hour of the experiment, after the administration of the drugs, about 46%, 50% and 60% of the maximum concentrations of citicoline released from the developed combination drug and Ceraxon were found in blood plasma, respectively. No citicoline was detected in blood plasma of any animals after 48 hours of the experiment. The obtained data in terms of the time to reach the maximum concentration of citicoline in blood plasma corresponds to those available in the literature: after oral and intravenous administrations of labeled citicoline to rats, its concentration reached its maximum value by 4–6 hours after the administration. It is most likely that the "concentration-time" curves of citicoline obtained characterize its residual content in blood plasma, because it is known from the literature (Secades 2016) that both intravenous and oral administrations of citicoline are rapidly metabolized to two main circulating metabolites: cytidine and choline.

To statistically assess the differences between the concentration values of memantine and citicoline in blood plasma after the administration of the developed drugs and reference drugs (Akatinol Memantine and Ceraxon), a two-tailed t-test for averages was used (the assessment was carried out at a confidence level of 95%). It was found that statistically significant differences in the concentrations of memantine in blood plasma of the rabbits after administration of the developed combination drug and Akatinol Memantine were determined at 4 points: 0.5; 1, 2 and 24 hours after their administration. When comparing the concentrations of citicoline in blood plasma after administration of Ceraxon and the developed combination drug, the differences in concentrations were statistically significant at all the sampling points.

The pharmacokinetic profiles of a pharmacological agent in blood when administered orally should be characterized by such parameters as maximum concentration (C_{max}), time to maximum observed concentration (T_{max}), area under curve (AUC), mean retention time (MRT), half-life ($T_{1/2}$) and rate of absorption (C_{max}/AUC_t) (Guidelines for Conducting Drug Preclinical Trials 2012). The pharmacokinetic parameters were calculated by a model-independent method – the method of statistical moments, because with the chamber access different pharmacokinetic parameters can be obtained, what can significantly influence on the results of studies.

Tables 5–8 contain individual and average values of the main pharmacokinetic parameters characterizing the bioavailability of memantine and citicoline from the developed combination drug and reference drugs Akatinol Memantine and Ceraxon.

Table 5. Pharmacokinetic Parameters of Memantine in Rabbit Plasma After a Single Pral Administration of the Developed Combination Drug

Mean	C_{max}, ng/ml	T_{max}, h	$AUC_{0\text{-}120}$, h×ng/ml	$AUC_{0\text{-}\infty}$, h×ng/ml	MRT, h	$T_{1/2}$, h	$C_{max}/AUC_{0\text{-}120}$, h^{-1}
Average	586.83	6.00	12946.00	15809.57	39.72	27.36	0.04
SD	219.36	1.26	2006.37	1823.70	15.69	12.87	0.01
Sx	89.55	0.52	819.10	744.52	6.41	5.06	0.01

Table 6. Pharmacokinetic Parameters of Memantine in Rabbit Plasma After a Single Oral Administration of Akatinol Memantine

Mean	C_{max}, ng/ml	T_{max}, h	AUC_{0-120}, h×ng/ml	$AUC_{0-\infty}$, h×ng/ml	*MRT*, h	$T_{1/2}$, h	C_{max}/AUC_{0-120}, h^{-1}
Average	314.00	5.67	9143.92	13869.04	68.50	47.42	0.03
SD	186.83	0.82	2880.88	2742.76	14.20	8.01	0.01
Sx	76.27	0.33	1176.11	1119.73	5.80	3.27	0.01

Table 7. Pharmacokinetic Parameters of Citicoline in Rabbits After a Single Oral Administration of the Developed Combination Drug

Mean	C_{max}, ng/ml	T_{max}, h	AUC_{0-120}, h×ng/ml	$AUC_{0-\infty}$, h×ng/ml	*MRT*, h	$T_{1/2}$, h	C_{max}/AUC_{0-120}, h^{-1}
Average	185.00	3.50	2792.96	5095.99	33.31	23.11	0.07
SD	74.00	1.76	1292.25	1622.68	10.06	7.55	0.02
Sx	30.21	0.72	527.56	662.46	4.11	3.08	0.01

Table 8. Pharmacokinetic Parameters of Citicoline in Rabbits After a Single Oral Administration of Ceraxon

Mean	C_{max}, ng/ml	T_{max}, h	AUC_{0-120}, h×ng/ml	$AUC_{0-\infty}$, h×ng/ml	*MRT*, h	$T_{1/2}$, h	C_{max}/AUC_{0-120}, h^{-1}
Average	80.83	4.33	1335.67	3995.64	63.41	43.45	0.06
SD	25.67	1.51	344.90	1350.50	40.58	27.88	0.01
Sx	10.48	0.61	140.81	551.34	16.57	11.38	0.01

Discussion

An analysis of the main pharmacokinetic data of memantine showed that the values of the maximum concentration (C_{max}) in blood plasma, calculated as the average of the highest measured value in each animal, are statistically significantly different when comparing the values obtained for Akatinol Memantine and for the developed combination drug. The variation coefficients for this indicator did not exceed 35% for the developed drug memantine and 54% for Akatinol Memantine.

The main parameter characterizing the bioavailability degree of the drug, AUC_{0-120}, had statistically significant differences between the values when comparing the developed combination drug and Akatinol Memantine. The variation coefficients of this parameter were 29% for Akatinol Memantine and 14% for the developed combination drug. There were no statistically significant differences for the T_{max} parameter for the studied drugs. The average time to reach the maximum concentration for Akatinol Memantine was 5.67±0.33 h, for the developed combination drug – 6.00±0.52 h. The variation coefficient of this parameter did not exceed 35% for all the drugs. The obtained results for this indicator are in line with the literature on the pharmacokinetics of Akatinol Memantine when taken orally by healthy volunteers: the T_{max} parameter was 5 hours.

The average retention time of memantine (*MRT*) had statistically significant differences for Akatinol Memantine and the developed combination drug. The parameter values were: for Akatinol Memantine – 68.50±5.80 h; and for the developed combination drug – 39.72±6.41 hours. The variation coefficient of this parameter was 19% for Akatinol Memantine and 36% for the developed combination drug.

Statistically significant differences in the half-life values ($T_{1/2}$) were also recorded for Akatinol Memantine and the developed combination drug. The parameter values were: for Akatinol Memantine – 47.42±3.27 h; and for the developed combination drug – 27.36±5.06 h. The variation coefficient of this parameter is 15% for Akatinol Memantine and 41% for the developed combination drug.

The C_{max}/AUC_{0-120} index characterizing the absorption rate showed no statistically significant differences. The parameter values were: 0.03±0.01 h^{-1} and 0.04±0.01 h^{-1}, respectively, for Akatinol Memantine and the developed combination drug. The relative bioavailability of memantine of the developed combination drug when compared to Akatinol Memantine was 114.0±10.7%.

An analysis of the basic pharmacokinetic data of citicoline showed that the values of the maximum concentration (C_{max}) in blood plasma, calculated as the average of the highest measured values in each animal, had statistically significant differences when comparing the developed combination drug and Ceraxon. The variation coefficient for this indicator hardly reached 37%.

The main parameter characterizing the degree of bioavailability of the drug, AUC_{0-24}, the same as in the case of the C_{max} parameter, had statistically significant differences when comparing the developed combination drug and Ceraxon. The variation coefficients for this parameter were 24% for Ceraxon and 29% for the developed combination drug.

There were no statistically significant differences for the T_{max} parameter for the studied drugs. The average time to reach the maximum concentration for Ceraxon was 4.33±0.61, for the developed combination drug – 3.50±0.72, respectively. The variation coefficients of this parameter were 32% and 46% for Ceraxon and the developed combination drug, respectively.

For the average retention time of citicoline (*MRT*), significant variability of the results was observed when comparing the values of this indicator obtained for all the studied drugs. For instance, the variation coefficients of this parameter were 58% for Ceraxon and 28% for the developed combination drug. The parameter values were the following: for Ceraxon –63.41±16.57 h; and for the developed combination drug – 33.31±4.11 h. There were no statistically significant differences for this value for the developed combination drug and Ceraxon.

There were also no statistically significant differences in the half-life values ($T_{1/2}$) for both studied citicoline drugs. The parameter values were the following: for Ceraxon – 43.45±11.38 h, and for the developed combination

drug – 23.11±3.08 h, respectively. The half-life was also characterized by a rather high variability. The variation coefficients for this parameter were 58% for Ceraxon, and 30% for the developed drug citicoline. The C_{max}/AUC_{0-120} value which characterizes the absorption rate, showed no statistically significant differences. The parameter values were 0.06±0.01 h^{-1} and 0.07±0.01 h^{-1}, respectively, for Ceraxon and the developed combination drug.

The relative bioavailability of citicoline from the developed combination drug when compared to Ceraxon was 127.5±24.2%.

Conclusions

Based on the results obtained on the concentrations of memantine and citicoline in bioassays, the main pharmacokinetic parameters of the studied drugs were calculated. It was found that 72 hours after the administration of memantine preparations, about 5% and 17% of the maximum concentration of memantine released from Akatinol Memantine and the developed combination drug were found in blood plasma, respectively. One hundred twenty hours after the beginning of the experiment, no memantine was detected in blood plasma of any animal. In comparison with Akatinol Memantine, statistically significant differences were observed in the developed combination drug in terms of C_{max}, AUC_{0-120}, MRT, and $T_{1/2}$. The relative bioavailability of memantine of the developed combination drug in comparison with that of Akatinol Memantine was 114.0±10.7%. Twenty-four hours after the beginning of the experiment, about 46% and 50% of the maximum concentrations of citicoline released from the developed combination drug and Ceraxon, respectively, were recorderd in blood plasma of the rabbits. In comparison with the pharmacokinetic parameters of Ceraxon, statistically significant differences were observed in the developed combination drug in terms of the following parameters: C_{max} and AUC_{0-120}. The relative bioavailability of citicoline from the developed combined drug in comparison with Ceraxon was 127.5±24.2%.

Conflict of interest

The authors declare no conflict of interests.

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