DOI: 10.15825/1995-1191-2021-2-114-121

COMPARATIVE ANALYSIS OF PHARMACOKINETIC PARAMETERS OF TRANSDERMAL AND INTRAMUSCULAR ADMINISTRATION OF GALAVIT®

E.G. Kuznetsova[1], O.M. Kuryleva[1], L.A. Salomatina[1], S.V. Kursakov[2], Z.Z. Gonikova[1], A.O. Nikolskaya[1], V.I. Sevastianov[1, 2]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Institute of Biomedical Research and Technology, Moscow, Russian Federation

Introduction. Immunomodulator Galavit® is a promising domestic drug for the prevention and treatment of various infectious diseases. Earlier, the authors have developed and investigated in vitro its new dosage form – transdermal therapeutic system (TTS). Positive results from experiments made it possible to proceed to the study of the pharmacokinetic parameters of Galavit® TTS in animals. **Objective:** to compare the pharmacokinetic parameters of intramuscular and transdermal administration of immunomodulator Galavit® in animal experiments. **Materials and methods.** Sodium aminodihydrophthalazinedione was used as a substance in the form of a powder to prepare a solution for intramuscular administration of 100 mg (trade name Galavit®, manufacturer SELVIM LLC). The pharmacokinetics of transdermal and intramuscular injections were studied in male Chinchilla rabbits weighing 4.5–5.0 kg. Serum sodium aminodihydrophthalazinedione concentrations in animals were determined by high-performance liquid chromatography using a specially developed technique. **Results.** In contrast to the injection method, a prolonged and uniform inflow of the drug substance (MP) into the body is observed for percutaneous administration of sodium aminodihydrophthalazinedione. The maximum serum Galavit® concentration for a 40 mg dose (0.172 ± 0.054 μg/mL) and for a 80 mg dose (1.16 ± 0.22 μg/mL) remained at a constant level for 9 and 8 hours, respectively. The relative bioavailability of the Galavit® transdermal therapeutic system was 0.65 and 1.06 for the same doses. **Conclusion.** Application of Galavit® 80 mg transdermal therapeutic system provides bioavailability that is similar to the intramuscular administration of this drug at the same dose. At the same time, its maximum serum concentration significantly decreases and the retention time of Galavit® in the body increases by more than 10 times, which can contribute to prolongation of the drug effect. Due to the current growing interest in the use of immunomodulator Galavit® for coronavirus infection COVID-19, the development and study of a new dosage form is a promising task.

Keywords: transdermal therapeutic system, sodium aminodihydrophthalazinedione, immunomodulator, pharmacokinetics.

INTRODUCTION

Over the past decades, scientific research in the development and implementation of highly active and competitive dosage forms in medical practice has been intensifying. More attention is being directed towards new systems and means for delivering medicinal substances with improved biopharmaceutical characteristics that increase therapeutic efficacy, tolerability and safety of drug therapy [1, 2]. One of these areas is the creation of transdermal therapeutic systems (TTS) – controlled-release dosage forms designed to continuously deliver the drugs they contain through intact skin into systemic circulation for a long (limited only by medical indications) time at a predetermined rate. The use of TTS increases the bioavailability of drugs, and also excludes the shortcomings of other methods by which it can be administered [3, 4].

Currently, the legitimacy and effectiveness of the use of immunotropic drugs that activate innate and adaptive immune systems for preventing and treating infectious diseases and a number of other diseases is widely discussed in the medical scientific literature [5].

A representative of this group of drugs is Russian synthetic drug Galavit® (aminodihydrophthalazinedione sodium is the active ingredient), which has immunomodulatory and pronounced anti-inflammatory properties.

Numerous studies have confirmed the efficacy of this drug in the complex immunocorrective therapy of patients with viral and bacterial pyoinflammatory diseases [6, 7]. For example, sodium aminodihydrophthalazinedione has been shown to reduce the severity of catarrhal and intoxication syndromes and significantly reduce duration in influenza diseases [8]. According to another study, this drug reduces the frequency and duration of

Corresponding author: Evgenia Kuznetsova. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (499) 196-26-61. E-mail: kuzeugenia@gmail.com

infection, it reduces the need for antibiotic therapy and normalizes the immune status in children with frequent acute respiratory viral infection [9].

The authors have previously developed and investigated in vitro a transdermal therapeutic system [10, 11] containing sodium aminodihydrophthalazinedione. The results of the experiments showed the fundamental possibility of transdermal drug transfer, which made it possible to proceed to the study of TTS Galavit® in animals in vivo.

Creation of dosage forms includes a number of mandatory steps, one of which is the study of pharmacokinetics. An experimental study of the pharmacokinetic parameters of a drug allows one to predict the plasma drug concentration, choose an approximate dosing regimen, which is then adjusted during clinical trials [12]. Various methods can be used to determine the plasma drug concentration, which reliably monitor the concentration of a pharmacological agent under the selected conditions of a pharmacokinetic experiment and meet the general requirements of selectivity, accuracy, and reproducibility. The most commonly used method is high-performance liquid chromatography (HPLC) [13–15].

The **objective** of this work is to compare the pharmacokinetic parameters of intramuscular and transdermal administration of the immunomodulator Galavit® in animal experiments.

MATERIALS AND METHODS

Materials

The substance was sodium aminodihydrophthalazinedione in the form of a powder for preparing a solution for intramuscular administration of 100 mg (trade name Galavit®, manufacturer SELVIM LLC). Its molecular weight is 206 Da.

In the manufacture of laboratory samples of Galavit®, auxiliary substances and materials approved for medical use, and which meets the requirements of the current regulatory documentation were used.

The microemulsion composition with Galavit® included the following components: purified water (FS.2.2.0019.18), 0.9% sodium chloride solution (ESCOM, Russia), sodium dodecyl sulfate (AppliChem Panreac, Spain), apricot kernel oil (Desert Whale Jojoba Company Ltd., USA), α-tocopherol acetate (BASF SE, Germany), docusate sodium (Sigma, USA), Decaglyn PR-20 emulsifier (Nikko Chemicals Co., Ltd, Japan).

To create TTS Galavit®, the following components were selected: elastic microspongy material Foam tape 9773 (3M, USA), sorbent base PALV-01 (Palma Group of Companies, Russia), and Skotchpak 9730 film (3M, USA).

The following reagents were also used: sodium citrate (NPO RENAM, Russia), potassium phosphate 2-substituted, 3-water (Panreac, Spain), potassium phosphate 1-substituted (PCGroup, Russia), potassium hydroxide (Panreac, Spain), acetonitrile for chromatography (Panreac, Spain), trifluoroacetic acid (Merck, Germany), syringe filters (celluloseacetate, 0.45 μm, 25 mm, Agilent Technologies, Germany).

Equipment

Equipment used: disperser Heidolph DIAX 900 (Germany), ultrasonic homogenizer HeilscherUIS250V, analytical balance (GH-200 AND, Japan), centrifuge Hettich Rotina 38R (Germany), liquid chromatograph Agilent 1200 (Agilent Technologies, USA), equipped with UV detector, autosampler, degasser and column oven.

Methodology for pharmacokinetic study of Galavit® with intramuscular administration and with the use of transdermal therapeutic system

The study of the pharmacokinetics of sodium aminodihydrophthalazinedione with transdermal and intramuscular administration was performed on male Chinchilla rabbits weighing 4.5–5.0 kg.

The rabbits were obtained from the laboratory animal nursery belonging to Krolinfo Ltd. The producer provided a veterinary certificate for the latest animal health control. All experimental animals were bred on purpose and had not previously participated in a study. Quarantine period was 14 days. All manipulations with the animals were carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123) Strasbourg, 1986).

The plasma concentration of sodium aminodihydrophthalazindione in rabbits during transdermal and intramuscular injections was studied according to the developed design. The animals were divided into four groups of 3 animals each. The first and second groups were injected once intramuscularly in doses of 40 and 80 mg; in the third and fourth groups, percutaneous administration of Galavit® in the same doses was studied.

TTS was applied to a pre-shaved area of the back skin at the base of the neck. The drug was glued to healthy skin no earlier than a day after the hair removal procedure.

Blood sampling of animals was performed before the drug was administered, as well as at discrete time intervals from the marginal ear vein into test tubes with 3.8% sodium citrate solution. Blood sampling times for TTS were 1, 2, 4, 6, 12, 15, 18, 20 and 24 hours of application. For the injectable form of the immunomodulator – 3, 6, 10, 20, 30, 40, 50 minutes, 1, 2, 3, 4, 5, 6, 7 hours after drug administration. Test tubes were centrifuged for 5 minutes at 1500 rpm, then plasma was carefully collected. The plasma levels of sodium aminodihydrophthalazinedione in the rabbits was measured by high-

performance liquid chromatography using a specially developed technique.

HPLC technique for quantifying plasma concentration of sodium aminodihydrophthalazinedione in the experimental animals

Sample preparation

Blood plasma was transferred into a 2.0 mL microcentrifuge tube and 200 µl of a 50% solution (by volume) of trifluoroacetic acid was added. The mixture was stirred on a vortex shaker for 2 minutes and centrifuged at 6000 rpm for 10 minutes. 500 µl of supernatant was transferred into a 1.5 mL HPLC microvial, 55 µL of a 50% potassium hydroxide solution (by weight) was added, and the mixture was stirred.

Chromatographic analysis

Chromatographic determination was performed on an Agilent 1200 liquid chromatograph under the following conditions:

Chromatographic column: Mediterranea Sea 18 25 × 0.46 cm, 5 µm (Teknokroma Analitica SA, Spain) with an 8 × 4 mm guard column filled with the same sorbent.

Mobile phase: acetonitrile: 0.015% solution (by volume) of trifluoroacetic acid, pH = 2.5 (15:85). The mobile phase was pre-filtered and degassed on a vacuum filtration device.

Mobile phase flow rate: 0.8 mL/min.

Elution mode: isocratic.

Column oven temperature: 25 °C.

Injected sample volume: 10 µL.

Detection: 221 nm.

Chromatography time: 16 min

Retention time: about 11.7 minutes.

Chromatograms were registered and processed using the ChemStation software (Agilent, USA). Results were statistically processed using Microsoft Office Excel 2003 software.

Lower limit of Galavit® quantification: 50.0 ng/mL.

Linearity range of the method: 50.0–2000 ng/mL.

Calculation of pharmacokinetic parameters

The pharmacokinetic method of study allows one to give a number of quantitative characteristics to absorption, metabolism (biotransformation), distribution and excretion of drugs from the body. For this, the following parameters were calculated:

- C_{max} – maximum plasma drug concentration (µg/mL).
- T_{max} – time to reach maximum drug concentration (h).
- AUC – total area under the plasma drug concentration-time curve from the moment it enters the body until complete elimination from the body (h·µg/mL).
- AUMC – total area under the curve of the product of time and plasma drug concentration from the moment it enters the body until its complete removal from the body (h^2·µg/mL).
- $T_{1/2}$ – drug elimination half-life – a period characterizing the rate of drug concentration decrease in the body fluids and tissues (h).
- MRT – mean residence time of drug in the body (h).
- β – elimination rate constant (h^{-1}).
- F – bioavailability. The relative bioavailability was determined by comparison with the bioavailability after intramuscular administration and was calculated using the formula:

$$F = \frac{AUC_{(TTS)} \times D_{(Injection)}}{AUC_{(Injection)} \times D_{(TTS)}},$$

where AUC is the area under the kinetic curve, D is the drug dose.

Pharmacokinetic parameters were estimated by a model-independent approach.

Statistical processing of results

Results were processed statistically in accordance with OFS.1.1.0013.15 "Statistical processing of chemical experiment results" using Microsoft Office Excel 2010 software. In addition, a two-sided Student's t-test was used [16]. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

A comparative analysis of pharmacokinetic parameters in transdermal and intramuscular injections of immunomodulator Galavit® in vivo was carried out.

The averaged pharmacokinetic curves of sodium aminodihydrophthalazinedione with TTC application and intramuscular injection at 40 mg and 80 mg dose are shown in Figs. 1 and 2, respectively.

As seen in Fig. 1, with intramuscular injection of 40 mg of sodium aminodihydrophthalazinedione, the maximum plasma concentration was reached after 10 minutes and was about 11.6 ± 0.9 µg/mL. At 30 minutes, there was a sharp 2-fold decrease in the plasma drug concentration. After 2 hours, it decreased to 0.324 ± 0.050 µg/mL, and after 4 hours it was below the quantification limit. With transdermal administration of the same dose of the drug, the plasma concentration of the immunomodulator increased slowly. By the fourth hour, the drug content in the blood was 0.123 ± 0.037 µg/mL. After 6 hours, it reached a maximum level of 0.172 ± 0.054 µg/mL and remained constant within the statistical error for the next 9 hours ($p > 0.05$). Further, we noted a gradual decrease in the plasma drug levels. By 24 hours of application, plasma concentration of sodium aminodihydrophthalazinedione in the animals was 0.099 ± 0.034 µg/mL.

When the dose of sodium aminodihydrophthalazindione was increased to 80 mg, the maximum plasma drug level after intramuscular injection increased 2-fold and was 23.2 ± 1.0 μg/mL by 10 minutes (Fig. 2). One hour after injection, there was a sharp drop to 3.82 ± 0.42 μg/mL. After 7 hours, concentration of the immunomodulator was below the quantification level. With percutaneous administration of Galavit®, the maximum concentration was 1.16 ± 0.22 μg/mL 6 hours after the start of application of the transdermal system. Note that from 4 to 12 hours of the study, the plasma drug concentration in the animals was almost constant ($p > 0.05$).

Thus, with percutaneous administration of sodium aminodihydrophthalazinedione, we observed prolonged and uniform flow of the drug substance into the blood, while maintaining its concentration in the blood at a constant level for 8–9 hours.

Note that with intramuscular administration of immunomodulator Galavit®, a 2-fold increase in the dose resulted in a 2-fold increase in the maximum plasma drug concentration. In the case of percutaneous administration, the same two-fold dose change caused a 6.7-fold increase in the maximum plasma drug level. Such an increase in the diffusion flux of the drug through the skin,

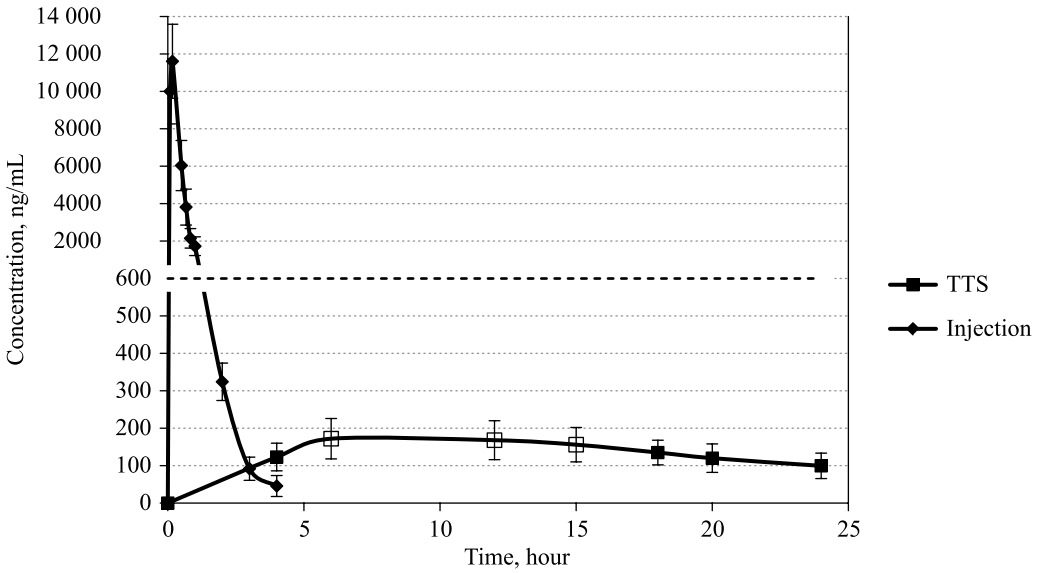

Fig. 1. Averaged dynamics of the concentration (±σ) of sodium aminodihydrophthalazinedione in the blood plasma of experimental animals with intramuscular and transdermal administration of a 40 mg dose. Differences in point values (□) are statistically insignificant ($p > 0.05$)

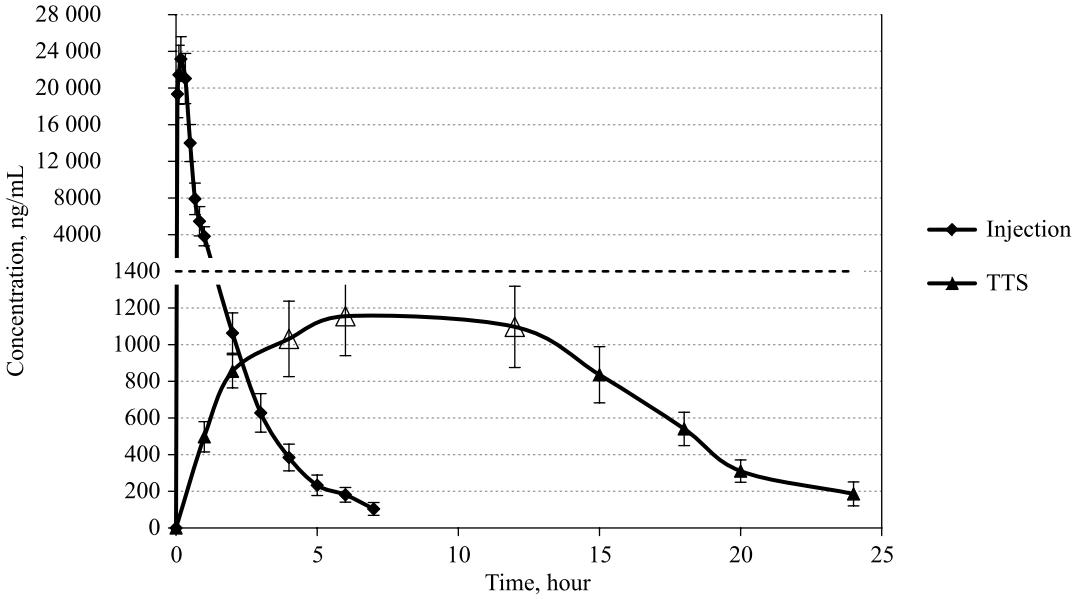

Fig. 2. Averaged dynamics of the concentration (±σ) of sodium aminodihydrophthalazinedione in the blood plasma of experimental animals with intramuscular and transdermal administration of a 80 mg dose. Differences in point values (Δ) are statistically insignificant ($p > 0.05$)

disproportionate to the increase in the drug content in TTS Galavit®, should be taken into account when selecting a therapeutic dose in a transdermal form.

The calculated pharmacokinetic parameters of sodium aminodihydrophthalazinedione at a single transdermal and intramuscular administration of two different doses to experimental animals are presented in Table.

Table

Pharmacokinetic parameters of sodium aminodihydrophthalazinedione in rabbits with transdermal and intramuscular administration

Parameters	Administration route, dose			
	Transdermal		Intramuscular	
	40 mg (n = 3)	80 mg (n = 3)	40 mg (n = 3)	80 mg (n = 3)
C_{max}, μg/mL	0.172	1.155	11.6	23.2
T_{max}, h	6	6	0.17	0.17
β, 1/h	0.0702	0.1686	2.74	1.81
$T_{1/2}$, h	9.8	4.6	0.25	0.38
AUC, h·μg/mL	4.7	18.6	7.21	17.54
AUMC, h^2·μg/mL	39.4	187.7	3.98	14.71
MRT, h	8.4	10.1	0.55	0.84

The decrease in sodium aminodihydrophthalazindione concentration in the blood after the steady-state period with TTC application was characterized by $T_{1/2}$ half-life, which was approximately 9.8 hours for a 40 mg dose and 4.6 hours for a 80 mg dose. The mean residence time of drug in the body (MRT) was approximately 8.4 hours and 10.1 hours for the lower and higher drug content in the TTS, respectively.

With intramuscular administration of the immunomodulator, the $T_{1/2}$ half-life was 0.25 hours and 0.38 hours, and the MRT was 0.55 hours and 0.84 hours for 40 mg and 80 mg doses.

Analyzing the results obtained, we can conclude that the use of a transdermal therapeutic system compared to intramuscular injection increases the mean residence time of drug in the body by more than 12–15 times. The half-life also increases by more than 10 times.

The calculated relative bioavailability of Galavit® transdermal therapeutic system was 0.65 for 40 mg and 1.06 for 80 mg. These results indicate that with an increase in the drug dose, the bioavailability of the transdermal therapeutic system becomes equal to the bioavailability of the immunomodulator when administered intramuscularly.

CONCLUSION

In the course of this work, the pharmacokinetics of intramuscular and transdermal administration of sodium aminodihydrophthalazinedione at 40 mg and 80 mg doses were studied in animals in vivo.

It was shown that application of Galavit® 80 mg transdermal therapeutic system provides a bioavailability that is equal to the bioavailability obtainable when this drug is administered intramuscularly at the same dose. Meanwhile, maximum plasma drug concentration decreases significantly and its residence time in the body increases by more than 10 times. This can help in prolonging the drug effect. Changes in drug concentration in the blood during application of TTS occurs gradually over several hours, in contrast to a sharp jump during intramuscular administration. This is an undisputed advantage of the Galavit® transdermal system in the case of long-term use for prophylaxis and supportive therapy.

It should be noted that at present, there is increased interest in the use of Galavit® in COVID-19 infection. Specifically, there was a recent report on the effectiveness of the drug in preventing moderate and severe forms of COVID-19 among medical workers under high risk of contracting SARS-COV-2 infection [17]. In this regard, the authors consider it promising to develop and study the domestic transdermal dosage form of immunomodulator Galavit®.

The authors declare no conflict of interest.

REFERENCES

1. *Sampiev AM, Nikiforova EB, Davitavjan NA.* Sovremennye dostizhenija v razrabotke i primenenii innovacionnyh lekarstvennyh sredstv. *Novye tehnologii*. 2012; 2: 247–254. [In Russ, English abstract].
2. *Leonova MV*. Novye lekarstvennye formy i sistemy dostavki lekarstvennyh sredstv: osobennosti peroral'nyh lekarstvennyh form. Chast' 1. *Lechebnoe delo.* 2009; 2: 21–31. [In Russ].
3. *Vasil'ev AE, Krasnjuk II, Ravikumar S, Tohmahchi VN.* Transdermal'nye terapevticheskie sistemy dostavki lekarstvennyh veshhestv (obzor). *Himiko-farmacevticheskij zhurnal*. 2001; 35: 29–42. [In Russ].
4. *Beregovyh VV, Pjatigorskaja NV, Prudkevich JuA, Kedik SA.* Transdermal'nye terapevticheskie sistemy dostavki lekarstvennyh sredstv. *Vestnik MITHT*, 2012; 7 (5): 17–22. [In Russ].
5. *Haitov RM.* Immunomoduljatory: mify i real'nost'. *Immunologija*. 2020; 41 (2): 101–106. [In Russ]. doi: 10.33029/0206-4952-2020-41-2-101-106.
6. *Djadina KS, Zemskov AM, Berezhnova TA, Mihajlova MD, Zemskova VA, Dobrosockih GV.* Perspektivy immunoterapii gnojno-vospalitel'nyh zabolevanij. *Vestnik novyh medicinskih tehnologij*. Jelektronnoe izdanie. 2020; 2: 81–89. [In Russ, English abstract]. doi: 10.24411/2075-4094-2020-16625.
7. *Luss LV, Martynov-Radushinskij AA*. Vtorichnaja immunnaja nedostatochnost'. Vsegda li nuzhny immunomoduljatory? *Medicinskij sovet*. 2014; 2: 40–45. [In Russ]. doi: 10.21518/2079-701X-2014-2-40-45.
8. *Sologub TV, Osinovec OJu*. Primenenie immunomodulirujushhego preparata galavit v kompleksnoj terapii grippa. *Klinicist.* 2012; 2: 76–80. [In Russ, English abstract].
9. *Rumjancev AG, Shherbina AJu.* Jeffektivnost' preparata galavit u chasto i dlitel'no bolejushhih detej starshe

6 let. *Rossijskij vestnik perinatologii i pediatrii*. 2008; 6: 100–101. [In Russ, English abstract].

10. *Kuznecova EG, Kuryleva OM, Salomatina LA, Sevast'janov VI.* Jeksperimental'noe issledovanie diffuzii immunomoduljatora Galavit® v model'noj sisteme. *Razrabotka i registracija lekarstvennyh sredstv*. 2020; 9 (1): 92–97. [In Russ, English abstract]. doi: 10.33380/2305-2066-2020-9-1-92-97.
11. *Kuznecova EG, Kuryleva OM, Salomatina LA, Sevast'janov VI.* Primenenie sinteticheskoj i biologicheskoj test-sistem pri razrabotke transdermal'nyh terapevticheskih sistem. *Perspektivnye materialy*. 2020; 8: 49–59. [In Russ, English abstract]. doi: 10.30791/1028-978X-2020-8-49-58.
12. Rukovodstvo po provedeniju doklinicheskih issledovanij lekarstvennyh sredstv. Chast' pervaja / Pod red. A.N. Mironova. M.: Grif i K, 2012. 944 s.
13. Farmacevticheskaja razrabotka: koncepcija i prakticheskie rekomendacii. Nauchno-prakticheskoe rukovodstvo dlja farmacevticheskoj otrasli. Pod red. S.N. Bykovskogo. M.: Pero, 2015.
14. *Zherdev VP, Voronina TA, Garibova TL, Kolyvanov GB, Litvin AA, Sariev AK i dr.* Ocenka farmakokinetiki i jeffektivnosti fenazepama u krys pri transdermal'nom i jenteral'nom sposobah vvedenija. Jeksperimental'naja i klinicheskaja farmakologija. 2003; 66 (1): 50–53. [In Russ].
15. *Narasimha Murthy S, Hiremath SR.* Clinical pharmacokinetic and pharmacodynamic evaluation of transdermal drug delivery systems of salbutamol sulfate. *Int J Pharm*. 2004; 287 (1–2): 47–53.
16. *Basok YB, Salomatina LA, Tikhobaeva AA, Sevastianov VI.* A Pharmacokinetic Study of Caffeine Transdermal Therapeutic Systems. *Advanced Metals, Ceramics and Composites* (ed. H. Tu, K. Solntsev, R. Zhou), Yunnan Publ. Group Corp., Kunming, China. 2013: 175–177.
17. *Sergienko VI, Bondareva IB.* Matematicheskaya statistika v klinicheskikh issledovaniyakh. M.: GEOTAR-MED, 2001: 256.
18. *Kolesov SV, Gorbatjuk DS, Panteleev AA, Bernakevich AI, Ukolov KJu*. Profilaktika srednih i tjazhelyh form COVID-19 aminodigidroftalazindionom natrija (Galavit®) u medicinskogo personala "krasnoj zony". *Immunologija.* 2020; 41 (6): 527–539. [In Russ]. doi: 10.33029/0206–4952-2020-41-6-527-539.

The article was submitted to the journal on 9.03.2021