Organ distribution of potential hypoglycemic drugs C7070

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ABSTRACT

Introduction: The organ distribution of a potential hypoglycemic drug - C7070 in rats was studied. **Materials and Methods:** The object of the study was the pharmacological substance C7070. The C7070 concentration was determined by high-performance liquid chromatography with mass spectrometer (MS)/MS detection. The measurement range was from 0.02 µg to 3876.00 µg in 1 ml of plasma or 1 g. Chromatographic separation was carried out on a 150 mm × 3.0 mm column of Zorbax Eclipse XDB C18 with a particle size of 3.0 µm (Agilent technology, USA). To obtain stable results for all analytical cycles, a protective membrane of Zorbax Eclipse XDB C18 (Agilent Technologies, USA) measuring 12.5 mm × 3.0 mm with a particle size of 5.0 µm at 40°C was used. Ballast proteins in test solutions were precipitated with acetonitrile, followed by extraction of the analyte with ultrasound. **Results and Discussions**: The drug is well distributed into organs. The greatest content of C7070 was observed in the tissues of the small intestine. The smallest content of C7070 was observed in muscle tissue and brain. The parameters obtained can be useful for clinical application and further studies of preparations C7070 on its basis.

KEY WORDS: C7070, Diabetes, High-performance liquid chromatography, Hypoglycemic agents, Organ distribution, Rat blood plasma

INTRODUCTION

World statistics showed that by the end of 2016, the number of people with diabetes is about 420 million people. According to the forecast-deputy International Diabetes Federation, by 2040, the number will increase to 642 million people. Considering this development of effective medicines for the treatment of diabetes is one of the urgent problems of modern medicine. Search for innovative molecules[1,2] is an important task of pharmacology. In this case, their research should be carried out for pharmacological purposes,[3,4] *in vivo*,[5,6] pharmacokinetic parameters[7] and clinical studies.[8,9]

One of the new developments in the treatment of diabetes is 3-(1H-benzimidazol-2-yl)-1,2,2-trimethylcyclopentanecarboxylic acid (C7070).[10] The structural formula is shown in Figure 1. Previously, we conducted pharmacokinetic studies in rats and rabbits.[11]

Interest in this substance - a potential hypoglycemic drug for the treatment of diabetes is due to the fact that antagonists of imidazoline receptors are antihypertensive drugs clonidine, moxonidine, and rilmenidine, as well as antidiabetic drug metformin. Antihypertensive preparations of clonidine, moxonidine, and rilmenidine in contrast to 3-(1H-benzimidazol-2-yl)-1,2,2-trimethylcyclopentanecarboxylic acid do not affect glycemic control. The antidiabetic drug metformin for safety (acute toxicity, lactic acidosis, and other side effects), the breadth of the therapeutic index, and the severity of antidiabetic properties is inferior to C7070. Unlike C7070, metformin has not been proven: The ability to restore the physiological function of the pancreatic β-cells, cerebroprotective, and nootropic effects.

Purpose

A study of the organ distribution of a potential hypoglycemic drug C7070 in rats.

Object

Pharmaceutical substance C7070: 12 rats (males weighing $350 \pm 20\%$ g) were included in the study. Adaptation time before the study was 8 days. In the study, animals were selected with no signs of abnormal appearance. The basic rules of maintenance and care are in accordance with the regulations given

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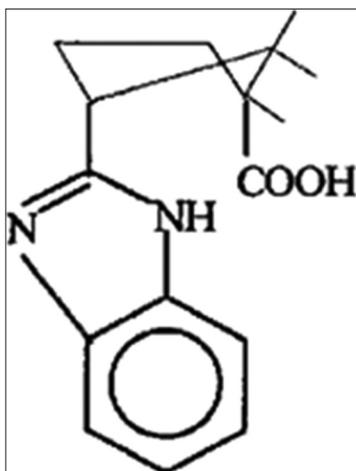
Figure 1: Structural formula C7070

in the manual guide for the care and use of laboratory animals. The National Academy Press – Washington, DC 2011.[12]

The following reagents were used in the work: Substance C7070 (CJSC VladMiVa, RF, Belgorod), fabomotizole (Sigma), formic acid (Panreac), ammonium acetate (Panreac), methanol (Merk), and acetonitrile for gradient chromatography (Merk), water 18.2 MΩ × cm, obtained with the Gene Pure system (Thermo Scientific, USA).

Methods of Research

To perform organ distribution studies, mass spectrometer (MS)/MS-high-performance liquid chromatography (HPLC) was developed to determine C7070 in rat blood plasma, organ homogenates and had full validation.[13] The methods corresponded to the requirements.[14-16]

The determination of C7070 in blood plasma, organ homogenates of rats was carried out on a liquid chromatograph UltiMate 3000 LC (Thermo Fisher Scientific, USA) equipped with a thermostatable automatic dispenser, vacuum degasser, gradient pump, and column thermostat. The analyte was detected on a Velos Pro mass spectrometer (Thermo Scientific, USA) with ionization in a heated electrospray (H-ESI-II). This equipment has already been used by us earlier[17] in quantitative studies and has shown good results.

Sample Preparation

The preparation of the initial solutions of C7070 included several steps. In the first stage, a stock solution of C7070 in methanol with a concentration of 0.2% was prepared. In the 2nd stage, C7070 solutions in methanol were prepared by dilution series to be added to standard solutions and quality control (QC) solutions with a concentration of 0.00002%, 0.002%, and 0.011%.

A solution of the internal standard was used at the same level of concentrations - 0.1% in methanol.

Calibration Solutions

The calibration solutions were prepared at seven concentration levels. Intact specimens of organs and tissues were used for the preparation of calibration solutions. 100 μl of intact final homogenate was placed in 1.5 ml Eppendorf tubes; aliquots of the starting solutions and 100 μl of the internal standard solution was added and mixed; and 0.1 ml of acetonitrile was added and mixed. Extracted C7070 in an ultrasound for 20 min after the sample was frozen at a temperature of −70°C. After thawing, it was centrifuged at 13,000 rpm and 4°C for 25 min. Thus, 14 solutions were prepared with seven levels of concentrations: 0.02 μg, 0.19 μg, 1.94 μg, 19.38 μg, 193.80 μg, 1938.00 μg, and 3876.00 μg in 1 ml plasma. Each level was prepared and analyzed twice.

QC Solutions

The QC solutions were prepared similarly to calibration solutions at two concentration levels in two replicates. Thus, 4 solutions were prepared with two levels of concentrations - 0.19 μg lower QC and 1938.00 (upper QC - UQC) in 1 ml of plasma.

Test Solution

A sample of tissue or organ was weighed, mixed in a container. An aliquot of water was added to the sample in an amount equivalent to the weight of the sample and homogenized. Next, the detectable substance was extracted from the organ tissues into the liquid phase of the homogenate by sonication for 20 min (final homogenate).

100 μl of the final homogenate was transferred to an Eppendorf tube of 1.5 ml capacity; 100 μl of internal solution was added and mixed; 0.1 ml of acetonitrile was added and mixed. Extraction of the analyte was performed on ultrasound for 20 min. The samples were frozen at a temperature of −70°C. After thawing, the samples were centrifuged at 13,000 rpm and 4°C for 15 min. The supernatant was analyzed.

Study Protocol

Organ distribution was studied after multiple intragastric (I.O) administration of C7070 to animals at doses of 25 mg/kg, 50 mg/kg. Whole blood, plasma, thymus, liver, brain, kidneys, heart, lungs, blood, intestines, spleen, skin, muscles, and fat tissue were collected.

Parameters HPLC-MS/MS

Chromatographic separation was carried out on a 150 mm × 3.0 mm column of Zorbax Eclipse XDB C18 (Agilent Technologies, USA) with a particle size

of 3.0 μm with a Zorbax Eclipse XDB C18 (Agilent Technologies, USA) protection column of 12.5 mm × 3.0 mm with a particle size of 5.0 μm, at a temperature of 40°C. The parameters of the chromatographic system are presented in Table 1.

RESULTS OF THE STUDY

The main distribution parameters were calculated on the basis of experimental results. Concentrations of C7070 in the investigated objects were calculated
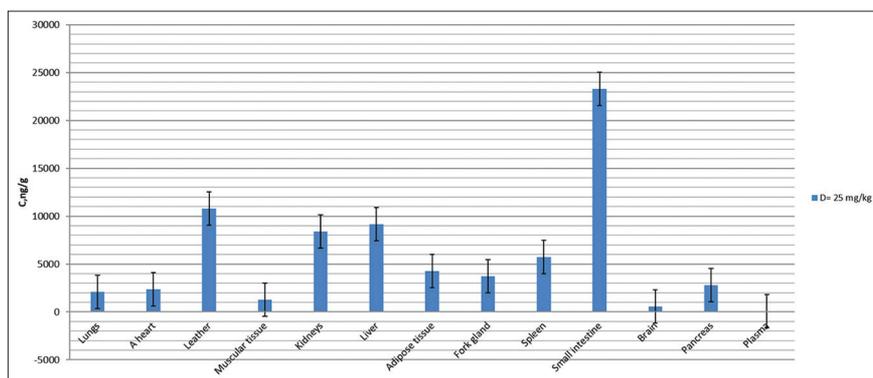

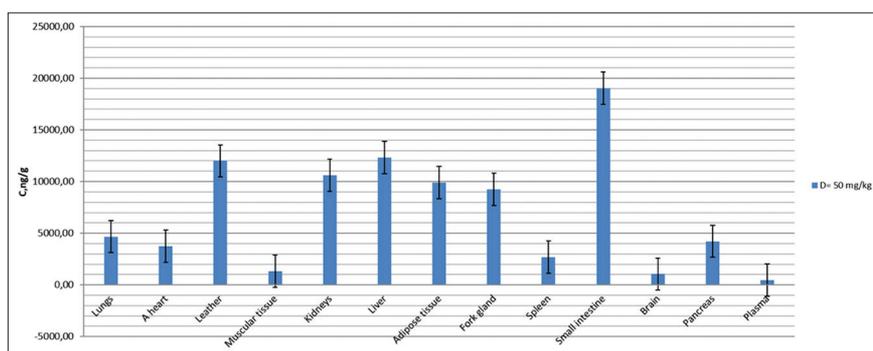**Figure 2:** The distribution diagram of C7070 in organs at a dose of 25 mg/kg

Figure 3: The distribution diagram of C7070 in organs at a dose of 50 mg/kg

Table 1: Parameters of the chromatographic system

UltiMate 3000 LC:					
Sample volume (μl):		2			
Mobile phase:		Gradient according to the following program:			
	Time, min	Flow, ml/min	5 mM ammonium acetate+0.1% formic acid	Acetonitrile, %	Methanol, %
	0	0.4	80	20	0
	5.0	0.4	80	20	0
	8.5	0.4	75	10	15
	9.0	0.4	20	70	10
	11.0	0.4	20	70	10
	11.1	0.4	80	20	0
	12.5	0.4	80	20	0
Retention times (min):	C7070 - about 4.7; The internal standard - about 8.5				
Injection time (min):	12.5				
Velos Pro:					
Tool:	Velos Pro (Thermo Scientific, США)				
Ionization type:	H-ESI				
Polarity:	C7070 «+» Internal standard «+»				
Mass transfer:	C7070-272.35→255.15; Internal standard – 307.41→114.				
Collision energy:	C7070-42; Internal standard – 19				
Voltage at source (V):	C7070-3000 Internal standard – 3000				
Source temperature (°C):	C7070-300 Internal standard – 300				
Capillary temperature (°C):	C7070-350 Internal standard – 350				
Sheath gas pressure (Arb):	C7070-60 Internal standard –60				
Aux gas pressure (Arb):	C7070-20 Internal standard – 20				

Table 2: The average Kd of C7070 for I.O administration to rats (n=6)

Test organ	Kd	
	Dose 25 mg/kg	Dose 5 mg/kg
Lungs	81.6±13.7	23.8±2.3
Heart	76.8±11.7	16.2±1.4
Leather	537.8±95.9	50.9±6.0
Muscular tissue	40.7±5.8	4.6±0.3
Kidneys	223.2±32.1	40.3±2.9
Liver	135.1±12.6	62.1±6.8
Adipose tissue	124.7±17.7	44.9±4.6
Fork gland	134.6±21.1	49.6±7.6
Spleen	355.9±67.0	14.7±1.7
Small intestine	310.9±29.2	123.6±22.0
Brain	10.4±1.0	3.7±4.0
Pancreas	86.1±12.2	14.2±1.0

from calibration curves. The sharply highlighted results (outliers) in animals at each time point were detected using the Grubbs statistical criterion.[18]

The distribution of C7070 in organs is shown in Figures 2 and 3.

The drug is well distributed into organs. The greatest content of C7070 was observed in the tissues of the small intestine. The smallest content of C7070 was observed in muscle tissue and brain.

Calculation of the apparent distribution coefficient (Kd) C70707 between plasma and tissues in rabbits with I.O administration is presented in Table 2.

The present data show that C7070 intensively penetrates into organs and tissues with a high level of hemocirculation. The greatest content of C7070 is characteristic of the small intestine, kidneys, skin, liver, and adipose tissue. The distribution of the drug in the lungs, heart, and spleen is close. A similar maximum concentration is characteristic for these organs. The smallest distribution of the drug C7070 is fixed for muscles and brain.

CONCLUSION

In the course of the work, it was established that the investigated drug substance C7070 is well absorbed from the gastrointestinal tract and distributed along the tissues. The highest content is observed in the small intestine, probably here is the systemic absorption. The experimental data of Kd show that the preparation intensively penetrates into the tissue (min = 3.7 ± 4.0, max = 537.8 ± 95.9). This indicator is of primary importance for hypoglycemic agents. The parameters obtained can be useful for clinical application and further studies of preparations C7070 on its basis.[19-21]

REFERENCES

1. Kravchenko DV, Beskhmelnitsyna EA, Korokin MV, Avtina TV, Sernov LN, Tishin AN, *et al.* Molecular screening of prospective candidates for TRPA1 ion channel selective antagonists. Res Result Pharmacol Clin Pharmacol 2016;1:63-6.
2. Bogus SK, Dukhanin AS, Kucheryavenko AF, Vinakov DV, Suzdalev KF, Galenko-Yaroshevsky PA. Pleyotropic antiaggregant effects of an innovative antiarrhythmic of Class III SS-68, an indole derivative. Res Result Pharmacol Clin Pharmacol 2017;3:3-13.
3. Ragulina VA, Kostina DA, Dovgan AP, Burda YE, Nadezhdin SV. Nuclear factor kappa B as a potential target for pharmacological correction endothelium-associated pathology. Res Result Pharmcol Clin Pharmacol 2017;3:114-24.
4. Danilenko LM, Klochkova GN, Kizilova IV, Korokin MV. Metabolic cardio protection: New concepts in implementation of cardio protective effects of meldonium. Res Result Pharmacol Clin Pharmacol 2016;2:95-100.
5. Molchanova OV, Pokrovskaya TG, Povetkin SV, Reznikov KM. Endothelio protective property of the combination of the thioctic acid and rosuvastatin shown in the endothelial dysfunction models. Res Result Pharmacol Clin Pharmacol 2016;2:9-15.
6. Shakhno EA, Savitskaya TA, Pokrovskaya TG, Yakushev VI, Pokrovskii MV, Grinshpan DD. Use of L-arginine immobilised on activated carbon for pharmacological correction of endothelial disfunction. Res Result Pharmacol Clin Pharmacol 2016;2:30-5.
7. Avdeeva NV, Kulikov AL, Pokrovskii MV, Avtina TV. Pharmacokinetic studies of new antiparkinsonian drug Rapitalam. Res Result Pharmacol Clin Pharmacol 2016;2:3-8.
8. Shakhno EA, Savitskaya TA, Pokrovskaya TG, Yakushev VI, Grinshpan DD. New dosage form of L-arginine based on its complex with cellulose acetate sulfate and activated carbon. Res Result Pharmacol Clin Pharmacol 2017;3:101-11.
9. Filippova OV, Malorodova TN, Pokrovskaya TG, Afanasiev YI. Pancreatogenic infections: Importance of microbiological monitoring and penetration of antimicrobial chemotherapeutic agents into the pancreas when defining therapeutic approach. Res Result Pharmacol Clin Pharmacol 2015;4:58-62.
10. Patent of the Russian Federation: IPC 7 C07D235/16, A61K31/4184, A61P3/10; Applicant and Patent Holder National Pharmaceutical Academy of Ukraine (UA), Merzlikin Sergey Ivanovich (UA) No. 2205826; Claimed. 2000132042/14, 21.12.2000; Publ. 10.06.2003.
11. Kulikov AL, Pokrovskii MV, Avtina TV, Galenko-Yaroshevskii PA, Povetkin SV, Reznikov KM, *et al.* Pharmacokinetic research of potential hygoglice-mic drugs C7070. Indo Am J Pharm Sci 2017;4:3688-94.
12. National Academy Press. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academy Press; 2011.
13. Buzov AA, Kulikov AL, Avtina TV, Pokrovskii MV, Osipova OA. Development and validation of methods of quantitative determination of the new antidiabetic drug in the blood plasma of rats by high performance liquid chromatography with mass spectrometric detection. Res Result Pharmacol Clin Pharmacol 2016;2:52-7.
14. Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Center for Drug Evolution and Research (CDER), US Government Printing Office. Washington, DC: Food and Drug Administration; 2013.
15. Mironov AN, Merkulov VA, Stepanov NN. In: Grif K, editor. Guidance on the Examination of Medicines. Vol. 1. Moscow: FDA; 2013. p. 328.
16. Guideline on Bioanalytical Method Validation. EMEA/CHMP/ EWP/192217/2009 Revelation 1 Corr. 2** Committee for Medicinal Products for Human Use (CHMP).
17. Avtina TV, Kulikov AL, Pokrovsky MV. Development and validation of the method of quantitative determination of imatinib in blood plasma by the method of high-performance liquid chromatography with mass spectrometry detection. Sci Result Series Med Pharm 2015;1:104-11.

18. Bland M. An Introduction to Medical Statistics. 3rd ed. Oxford: Oxford Medical Publications; 2000. p. 422.
19. Artursson P, Karlsson J. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem Biophys Res Commun 1991;175:880-5.
20. Musther H, Olivares-Morales A, Hatley OJ, Liu B, Rostami Hodjegan A. Animal versus human oral drug bioavailability: Do they correlate? Eur J Pharm Sci 2014;57:280-91.
21. Rojas Gómez R, Restrepo Valencia P. *In vitro-in vivo* pharmacokinetic correlation model for quality assurance of antiretroviral drugs. Colomb Med (Cali) 2015;46:109-16.