Research Article

Tissue distribution of potential antidiabetic agent C7070

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ABSTRACT

Background: C7070 is a novel imidazoline receptors agonist for the treatment of diabetes mellitus. **Materials and Methods:** The study was included 12 male Wistar rats. The C7070 concentration was determined by high-performance liquid chromatography with tandem mass spectrometry 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 Technologies, 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 Discussion:** 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 contain specific pharmacological activity,[3-7] including *in vivo*[8-12] and *in vitro*[13] studies, toxicological,[14] clinical, and pharmacokinetic studies.[15]

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.[16]

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Interest in this substance-a potential hypoglycemic drug for the treatment of diabetes is due to the fact that agonists of imidazoline receptors are antihypertensive drugs clonidine, moxonidine, and rilmenidine, as well as antidiabetic drug metformin. Anti-hypertensive drugs 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 are 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.

MATERIALS AND METHODS

The object of the current study was pharmaceutical substance C7070. Twelve rats (males weighing 350 ± 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

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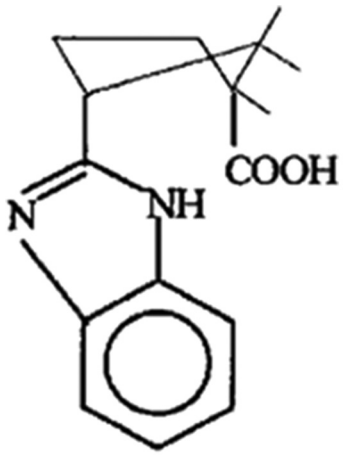

Figure 1: Structural formula C7070

with the regulations given in the Manual Guide for the care and use of laboratory animals. The National Academies Press. – Washington, D. C. 2011.[17]

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

To perform organ distribution studies, tandem mass spectrometry (MS/MS) high-performance liquid chromatography (HPLC) was developed to determine C7070 in rat blood plasma, organ homogenates and had full validation.[18] The methods corresponded to the requirements of Buzov et al.[19] Guidance for Industry: Bioanalytical Method Validation,[20] Mironov et al.[21]

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 thermostable 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[22] 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 second 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 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, mixed, 0.1 ml of acetonitrile added, and mixed extracted C7070 in 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 were prepared similarly to calibration solutions at two concentration levels in two replicates. Thus, four solutions were prepared with two levels of concentrations −0.19 µg lower QC and 1938.00 upper QC 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, mixed, 0.1 ml of acetonitrile added, and mixed. The 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 and 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

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				

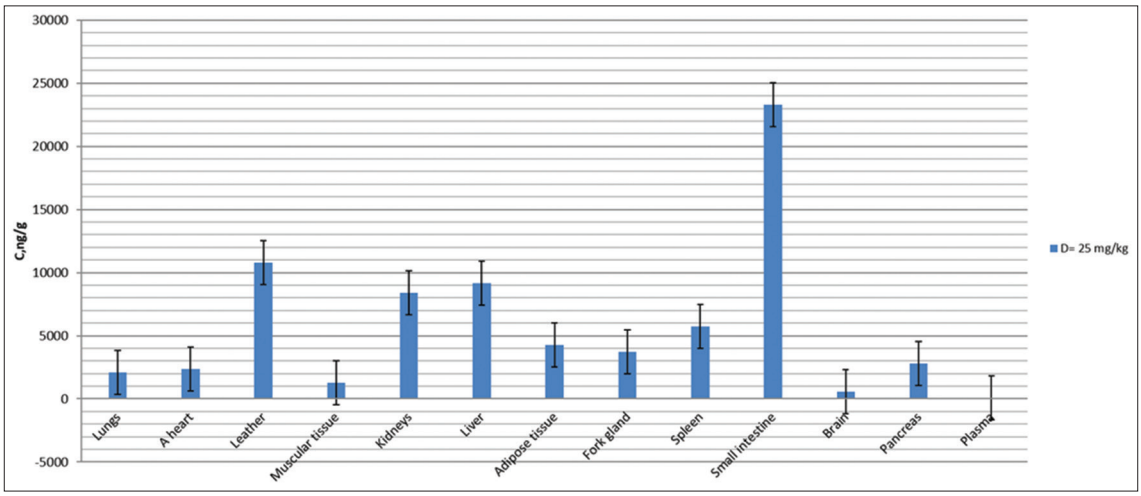

Figure 2: The distribution diagram of C7070 in organs at a dose of 25 mg/kg

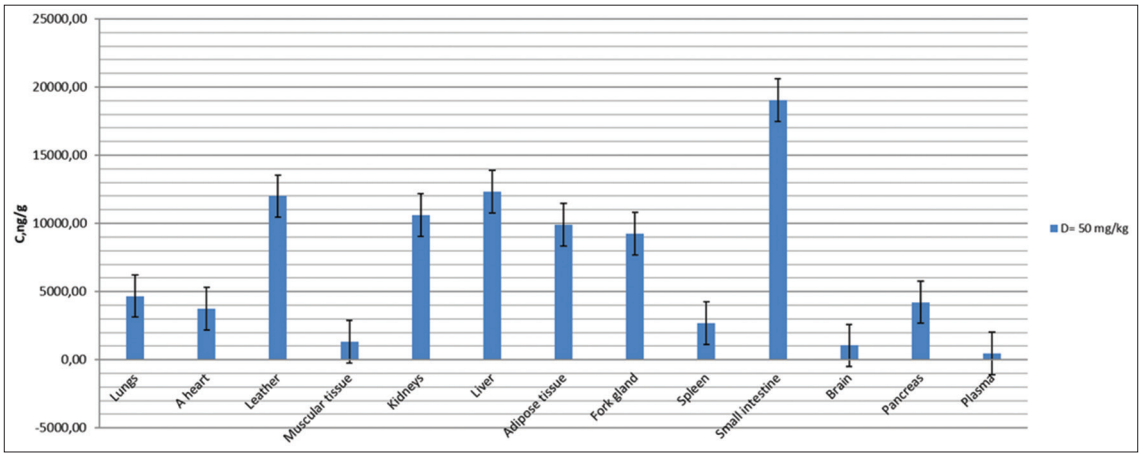

Figure 3: The distribution diagram of C7070 in organs at a dose of 50 mg/kg

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

Test tissue	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

of 40°C. The parameters of the chromatographic system are presented in Table 1.

RESULTS

The main distribution parameters were calculated on the basis of experimental results. Concentrations of C7070 in the investigated objects were calculated from calibration curves. The sharply highlighted results (outliers) in animals at each time point were detected using the Grubbs statistical criterion.[23]

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 of 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 with 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.[24-27]

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