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PHARMACOKINETIC RESEARCH OF POTENTIAL HYGOGLICEMIC DRUGS C7070

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Abstract:

Introduction: *The development of effective drugs for the treatment of diabetes is one of the urgent problems of modern medicine; we conducted pharmacokinetic studies of the innovative hypoglycemic drug - C7070, in rabbits and rats.*

Materials and Methods: *The object of the study was substance C7070. Two methods of administration have been studied: intravenously and intragastrically. The concentration of C7070 is determined in blood plasma by a sensitive and selective HPLC method with tandem mass spectrometry detection. The range of detection was from 0.02 μg to 3876.00 μg in 1 ml of plasma in the animals under study. Chromatographic separation was performed on a 150 × 3.0 mm column of Zorbax Eclipce XDB C18 with a particle size of 3.0 μm (Agilent technologies, USA). To obtain stable results, a Zorbax Eclipce XDB C18 (Agilent technologies, USA) protection column of 12.5 × 3.0 mm with a particle size of 5.0 μm was used at 40 ° C for all analytical cycles. Ballast proteins in the test solutions were precipitated with acetonitrile followed by extraction of the analyte with ultrasound.*

Results and its Discussion: *With intragastric administration, the maximum concentration (C_{max}) of C7070 in blood plasma reached, on average, in rabbits through (t_{max}) 60 ± 0.1 minutes, in rats after 170.0 ± 79.8 minutes and was 34.6 ± 7.3 μg/ml and 17.6 ± 1.4 μg/ml, respectively. The half-life ($t_{1/2}$) was prolonged and was 291.8 ± 17.1 minutes for rabbits and 225.2 ± 12.4 minutes for rats. The absolute bioavailability (f_a) of C7070 in rabbits was 78.2 ± 1.0%, in rats 18.1 ± 2.0%. When administered intravenously, C_{max} C7070 in blood plasma averaged 123.1 ± 23.7 μg/ml in rabbits and 337.6 ± 40.5 μg/ml in rats. The half-life period ($t_{1/2}$) was prolonged and amounted to 225.5 ± 15.9 minutes for rabbits and 154.1 ± 5.1 minutes for rats.*

The Conclusion: *The pharmacokinetic characteristics of a potential hypoglycemic drug C7070 in animals (rats, rabbits) under two routes of administration, intra-gastrointestinal and intravenous, were studied. The parameters obtained can be useful for clinical application and further studies of C7070 drugs based on it.*

Key words: *C7070, diabetes mellitus, hypoglycemic agents, blood plasma of rabbits and rats, high-performance liquid chromatography, pharmacokinetics.*

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INTRODUCTION:

According to the world statistics, over the past 10 years the number of people with diabetes mellitus (DM) has more than doubled and by the end of 2016 it is about 420 million people. According to the forecasts of the International Diabetes Federation, by the year 2040, 642 million people will suffer from diabetes.

Taking this into account, the development of effective medicines for the treatment of diabetes is one of the urgent problems of modern medicine.

The search for innovative molecules [0, 2] is an important task of pharmacology. In this case, their study should be carried out on pharmacological targets [0, 0], in vivo models [0, 0], pharmacokinetic parameters [0] and clinical studies [0, 0].

One of the new developments in the treatment of diabetes is 3- (1H-benzimidazol-2-yl) -1.2.2-trimethylcyclopentanecarboxylic acid (C7070) [0].

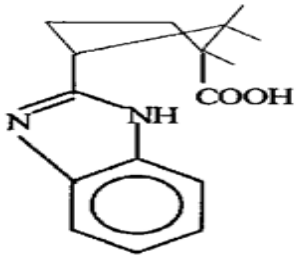

Fig 1: Structural formula C7070

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 an antidiabetic drug metformin. Antihypertensive drugs clonidine, moxonidine and rilmenidine unlike 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 pancreatic β-cells, cerebroprotective and nootropic effects.

The purpose of research - the study of the pharmacokinetics of potential hypoglycemic drug C7070 on animals (rats, rabbits). Материалы и методы исследования.

The object of study - is substance C7070. Twelve rabbits (males weighing 3500 ± 20% g) and 12 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 with the regulations given in the manual Guide for the care and use of laboratory animals. The National Academy press. – Washington, D.C. 2011 [0].

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

Methods of research. To carry out pharmacokinetic studies, HPLC MS/MS was developed to determine the C7070 in the blood plasma of rats and rabbits and complete validation [0]. Methods of keeping up with modern requirements [0, **Error! Reference source not found.**, 0].

The determination of C7070 in blood plasma of rats and rabbits was carried out on a liquid chromatograph UltiMate 3000 LC (Thermo Fisher Scientific, USA) equipped with a thermostatable automatic dispenser, vacuum degasser, gradient pump, 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 [**Error! Reference source not found.**] 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 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. 100 µl of plasma were placed in 1.5 ml Eppendorf tubes, aliquots of the starting solutions and 100 µl of the internal standard solution were added, mixed, 0.1 ml of acetonitrile added, and mixed. Extracted C7070 in an ultrasonic bath for three minutes. 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 minutes. 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.

Quality control solutions (QC) 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 quality control - LQC) and 1938.00 (upper quality control - UQC) in 1 ml of plasma.
The test solutions were prepared similarly to the calibration solutions. 100 μl of plasma was placed in 1.5 ml Eppendorf tubes, 100 μl of internal standard solution was added, mixed, 0.1 ml of acetonitrile added, and mixed. Extracted C7070 in an ultrasonic bath for three minutes. The samples were frozen at a temperature of -70 ° C. After thawing, the samples were centrifuged at 13,000 rpm and 4 ° C for 25 minutes.
Parameters HPLC-MS/MS. Chromatographic separation was carried out on a 150 mm × 3.0 mm column of Zorbax Eclipce XDB C18 (Agilent technologies, USA) with a particle size of 3.0 μm with a Zorbax Eclipce XDB C18 (Agilent technologies, USA) protection column of 12.5 × 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.

Table 1: Parameters of the chromatographic system

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

Protocol for the study of the pharmacokinetics of C7070 in rabbits. Twelve rabbits were pre-catheterized into the right ear vein. Twelve hours before the experiment began, the animals were deprived of food, leaving free access to water. On the third day after the catheterization, the test substance was administered.

With intravenous dosing, the test substance was administered bolus to six rabbits in the ear vein as a solution of 26.7 mg/ml in propylene glycol at a dose of 26.7 mg/kg. Blood was collected through a catheter in a volume of 0.3 ml into polypropylene tubes containing 20 µl of 5% EDTA before injection, at 5, 15, 30, 60, 120, 240, 480, 1440 minutes after administration. With intragastric dosing, the substance was administered with a probe in the form of a solution of 26.7 mg/ml in propylene glycol at a dose of 26.7 mg/kg. Blood was collected through a catheter in a volume of 0.3 ml into polypropylene tubes containing 20 µl of 5% EDTA before injection, at 15, 30, 60, 120, 240, 480, 1440 minutes after administration. The blood plasma was separated by centrifugation at 5600 g for 10 min and stored until analysis at a temperature of -70 ° C.

Protocol for the study of the pharmacokinetics of C7070 in rats. Twelve rats were pre-catheterized into the right jugular vein. For the operation, intraperitoneal anesthesia was used: 10% zolletil: 2% xylazine = 1: 5, 70 µl/100 g of body weight. Twelve hours before the experiment began, the animals were deprived of food, leaving free access to water.

With intravenous dosing, the test substance was administered bolus to six rats in the tail vein as a 50 mg/ml solution in propylene glycol at a dose of 50 mg/kg. Blood was collected through a catheter in a volume of 0.2 ml into polypropylene tubes containing 20 µl of 5% EDTA before injection, at 5, 15, 30, 60, 120, 240, 480, 1440 minutes after administration. With intragastric dosing, the substance was administered with a probe in the form of a solution of 50.0 mg/ml in propylene glycol at a dose of 50 mg/kg. Blood was taken through a 0.2 ml catheter into polypropylene tubes containing 20 µl of 5% EDTA before injection, at 15, 30, 60, 120, 240, 480, 1440 minutes after administration. The blood plasma was separated by centrifugation at 5600 g for 10 min and stored until analysis at a temperature of -70 ° C.

RESULTS OF THE STUDY:

The main pharmacokinetic parameters were calculated on the basis of experimentally obtained data. 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 [0].

Pharmacokinetics of C7070 in rabbits. Figure 2 shows the average pharmacokinetic profiles of C7070 for intragastric and intravenous routes of administration to rabbits (n = 6) at a dose of 26.7 mg/kg.

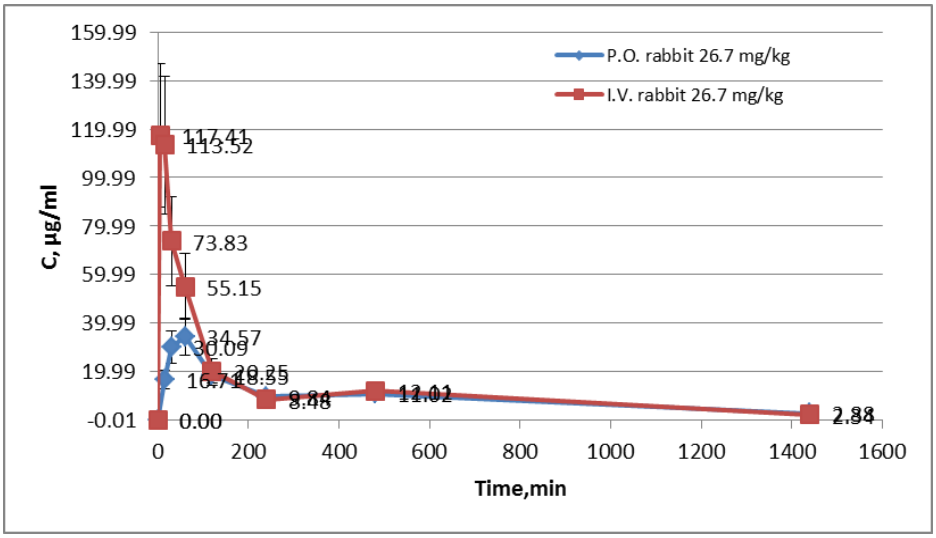

Fig 2: The average concentration-time dependence for two routes of administration of C7070 intragastric (P.O.) and intravenous (I.V.) in a dose of 26.7 mg/kg in rabbits

Table 2 presents the main pharmacokinetic characteristics of C7070 obtained when the drug was administered intragastrically and intravenously to rabbits (n = 6) at a dose of 26.7 mg/kg.

Table 2: The average pharmacokinetic characteristics of C7070 for intragastric and intravenous administration to rabbits (n = 6) at a dose of 26.7 mg/kg.

Indicator	The route of administration	
	P.O.	I.V.
$AUC_{(0-1440)}$, (μg/ml) × min	14113.4±1916.9	18179.0 ±1037.5
$AUC_{(0-\infty)}$,(μg/ml) × min	15326.5±1840.4	18946.7±1122.7
C_{max}, μg/ml	34.6±7.3	123.1±23.7
t_{max}, min	60±0.1	-
$t_{1/2}$, min	291.8±17.1	225.5±15.9
MRT, min	421.0±24.7	325.3±23.0
f_a, %	78.2±1.0	-

The results show that, with intragastric administration, the maximum concentration (C_{max}) of C7070 in rabbit blood plasma reached an average of 60 ± 0.1 minutes and was 34.6 ± 7.3 μg/ml. The area under the pharmacokinetic curve ($AUC_{(0-1440)}$) from the time of entry to the time of the last blood sampling was 14113.4 ± 1916.9 (μg/ml) × min for intragastric administration and 18179.0 ± 1037.5 (μg/ml) × min for intravenous administration. The half-life period ($t_{1/2}$) was prolonged and was 291.8 ± 17.1 minutes for intragastric introduction, 225.5 ± 15.9 minutes for intravenous administration. The mean retention time (MRT) of C7070 was 421.0 ± 24.7 minutes for intragastric administration and 325.3 ± 23.0 minutes for intravenous administration. The absolute bioavailability (f_a) of C7070 for intragastric dosing in rabbits was 78.2 ± 1.0 %.

Pharmacokinetics in rats. Figure 3 shows the average pharmacokinetic profiles of C7070 for intragastric and intravenous routes of administration to rats (n = 6) at a dose of 50.0 mg/kg.

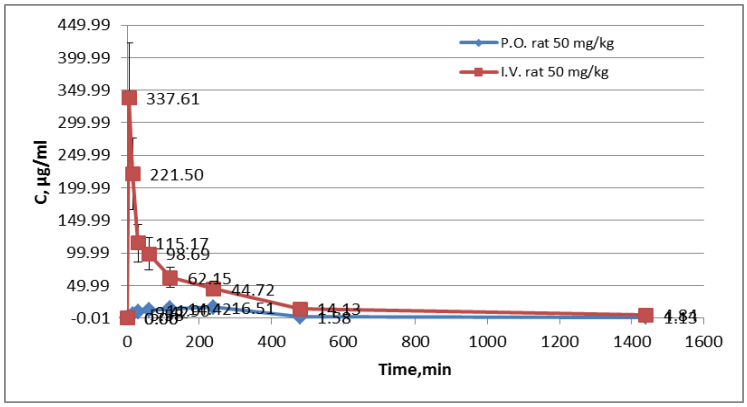

Fig 3: The average concentration-time dependence for two routes of administration of C7070 intragastric (P.O.) and intravenous (I.V.) in a dose of 50.0 mg/kg in rats

Table 3 presents the main pharmacokinetic characteristics of C7070 obtained by administering the drug intragastrointestinal and intravenously to rats (n = 6) at a dose of 50.0 mg/kg.

Table 3: The average pharmacokinetic characteristics of C7070 for intragastric and intravenous administration to rats (n = 6) at a dose of 50.0 mg / kg

Indicator	The route of administration	
	P.O.	I.V.
$AUC_{(0-1440)}$, (μg/ml) × min	6592.7±577.3	36777.1± 2888.9
$AUC_{(0-\infty)}$,(μg/ml) × min	6963.5±622.7	37857.7± 3180.1
C_{max}, μg/ml	17.6±1.4	337.6± 40.5
t_{max}, min	170.0±79.8	-
$t_{1/2}$, min	225.2±12.4	154.1±5.1
MRT, min	325.0±17.9	222.3±7.3
f_a, %	18.1±2.0	-

The results show that with intragastric administration of C_{max} C7070 in rat blood plasma averaged 170.0 ± 79.8 minutes and was 17.6 ± 1.4 μg/ml. AUC $_{(0-1440)}$ from the time of entry to the time of the last blood sampling was 6592.7 ± 577.3 (μg/ ml) × min for intragastric administration and 36777.1 ± 2888.9 (μg/ml) × min for intravenous administration. $t_{1/2}$ continuous, was 291.8 ± 17.1 minutes for intragastric administration and 225.5 ± 15.9 minutes for intravenous administration. MRT C7070 was 421.0 ± 24.7 minutes for intragastric administration and 325.3 ± 23.0 minutes for intravenous administration. The absolute bioavailability (f_a) of C7070 for intragastric dosing in rabbits was 78.2 ± 1.0%.

Analysis of these results shows that the C7070 is well absorbed from the gastrointestinal tract, retained in the body for a long time. From the point of view of developing a new antidiabetic drug, this is an undoubted plus. There is the possibility of using a simple and affordable dosage form for oral administration with a pronounced therapeutic effect for a long time.

It is worth noting that in the course of the work, there were interspecies differences in pharmacokinetic parameters with intragastric dosing. Of the many alleged causes, it is necessary to identify the mechanisms of absorption in the body. According to the physical and chemical properties of C7070 has a poor solubility in water. It is well known that this is the basic barrier rate of absorption for such a drug. For most drugs, absorption occurs through passive transcellular and paracellular transport, limited due to tight contacts between cells. Absorption occurs in a thin layer of intestinal epithelial cells, which covers the luminal surface of the intestinal wall. Thus, the speed and completeness of the absorption will directly hang from the surface area [0]. With such results, it can be assumed that interspecies differences are associated with the difference in the areas of the surface of the intestinal wall in rabbit and rat. However, variations within one species of animals have low values (not more than 20%). This suggests that when a person is dosed, the result will be predictable [0, 0].

CONCLUSION:

In the course of the work, the pharmacokinetic characteristics of the potential hypoglycemic drug C7070 in animals (rats, rabbits) were successfully studied by two routes of administration, intragastric and intravenous. It was found that the drug is well absorbed from the gastrointestinal tract, it is well distributed into tissues and is excreted unchanged. The parameters obtained can be useful for clinical application and further studies of preparations C7070 on its basis.

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