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PHARMACOKINETIC STUDIES DERIVED INDOLE SS-68 WITH ANTIARRHYTHMIC AND ANTIANGINAL PROPERTIES

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Abstract
A method of quantitative determination of SS-68 derivative of indole in rabbit blood plasma by high performance liquid chromatography with tandem mass spectrometric detector (HPLC-MS/MS). Conducted pharmacokinetic studies SS-68 in the body of rabbits. Set the main pharmacokinetic parameters of the substance that allow you to optimize the future use of it's as a potential drug in cardiology practice.
Keywords: compound SS-68, HPLC-MS/MS, pharmacokinetic studies, plasma of rabbits

Introduction

In previously studies was shown that compound SS-68 has a preventive and stopping action in case of cardio-neurogenesis rhythm disorders of heart outweighing the activity of number traditional and modern antiarrhythmic drugs [1-8]. High antiarrhythmic efficacy of SS-68 is connected with the blocking of ion currents (in maximal to acetylcholine-depended K^+ current, Ca^{2+} L-type current and fast K^+ current of the detained straightening), choline- and adrenoreceprores of cardiomiocites and neurons [8-11].

In conditions of intact and ischemized myocardium SS-68 increases a volume rate of coronary blood flow, makes an oxygen reserve in myocardium, decreases a blood pressure, slows a heartbeat, increases collateral blood circulation in ischemic focus, decreases a oxygen consumption by myocardium [12-14]. Antianginal SS-68 properties are due to blocking of pacemaker cells If- [15] and β1-adrenoreceptors. Coronary- and vasodelatating (peripheral arteries) activity of SS-68 is combined with activating at ATP-sensitive K^+ canals of smooth muscle cells (SMC) of coronal vessels in the first case, and with the increasing of a total exiting K^+ current, entailing the hyperpolarization activated SMC arteries, which leads to the blockade of Ca^{2+}-potential controlled channels and relaxation of SMC [6].

In addition, SS-68 has pleiotropic properties: a bronchodilator (due to β2-adrenomimetic action), antithrombotic (antiplatelet) and anti-inflammatory [6, 9] actions.

The above data indicate the feasibility of further preclinical study SS-68 to create on its basis drugs having antiarrhythmic and antianginal effects.

Experimental part

Reagents used in this study are the follows: fabomotizole (Sigma), formic acid (Panreac), ammonium acetate (Panreac), methanol (Merk) acetonitrile for gradient chromatography (Merk), water purified and deionized with "Gene Pure" system (Thermo Scientific, USA). The method of synthesis of compounds SS-68 developed and the necessary quantity acquired within the framework of the state task of the Ministry of education and science

of the Russian Federation No. 4.129.2014 in the Department of chemistry of natural and macromolecular compounds, faculty of chemistry of southern Federal University.

The definition of SS-68 in the blood plasma of rabbits was carried out with a liquid chromatograph UltiMate 3000 LC (Thermo Fisher Scientific, USA) equipped with a thermostated automatic dispenser, vacuum degasser, gradient pump, and column thermostat. Detection of the analyte was carried out with a mass-spectrometer

Velos Pro (Thermo Scientific, USA) under ionization in the heated electrospray (H-ESI-II).

Instruments and UHPLC–MS/MS Conditions

Chromatographic separation was performed on a column size of 150 × 3.0 mm, filled by reversed-phase sorbent Eclipce Zorbax XDB C18 with particle size 3.0 mm with guard column Zorbax XDB C18 Eclipce of 12.5×3.0 mm with a particle size of 5.0 μm at a temperature of 40 °C in the mode of linear gradient of eluent at a flow rate of 0.4 ml/min by the following program:

– the stage of separation: eluent A (5 mM Ammonium acetate + 0.1% formic acid) to 55% → 45%; eluent B (acetonitrile) to 45% → 55% in 4 minutes;

- the washing step: eluent A (5 mM Ammonium acetate + 0.1% formic acid) to 45%; eluent B (acetonitrile) – 55% – 0,5 minutes;

– stage equilibration: eluent A (5 mM Ammonium acetate + 0.1% formic acid) – 55%; eluent B (acetonitrile) to 45% in 2 minutes.

The volume of injected sample was 5 μl. Approximate retention times under these conditions: SS-68 – about 3.7 min; internal standard (fabomotizole) – about 2.1 min. Injection time of 7.0 min. Ionization was performed with H-ESI in the positive -ion mode. Scanning was performed by selectively ion monitoring (SIM). The precursor-product ion transitions for SS-68 – 305,26→208,0, internal standard (fabomotizole) – 307,41→114,0. The collision energy for SS-68 – 43, for the internal standard (fabomotizole) – 30. The voltage at the source is 3000 V. The source temperature 300 °C. The temperature of the capillary 350°C. The sheath gas pressure 60 Arb. Aux gas pressure of 20 Arb.

Sample preparation.

In accordance with modern requirements for bioanalytical methods [16, 17, 18] prepared standard solutions with different concentrations. Preparation of solutions SS-68 included several stages. The first stage was preparing the stock solution SS-68 in methanol with a concentration 0,080 %. In a second step by a series of dilutions prepared solutions SS-68 in methanol to be added to the standard solutions and solutions of quality control with a concentration of 0,00080 %, 0,0000080%. Solution internal standard (fabomotizole) used on one level, the concentration in methanol 0,0008 % to add to the subjects, standard and test solutions.

Solutions to create a calibration curve prepared in seven concentrations. For this, 100 μl of plasma was placed in 1.5 ml Eppendorf , then added aliquots of stock solutions and 100 μl of internal standard, mixed, added 100 μl of acetonitrile, mixed. Then we carried out the extraction of the analyte by vortex for 3 minutes. After extracting the samples were centrifuged at 13000 rpm and a temperature of 4 ºC for 25 minutes. The supernatant was decanted and analyzed. Thus were prepared solutions of 14 with seven concentrations – 8,02 ng; 80,2 ng; 802,0 ng; 1604,0 ng; 2406,0 ng; 4010,0 ng; and 8020,0 ng in 1 ml of plasma of rabbits. Each level was prepared and analyzed twice.

Solutions quality control (QC) were prepared similarly solutions to create a calibration curve at four levels of concentrations – 8,02 ng (lower limit of quantification – LLOQ), 80,2 ng (lower quality control – LQC), 1604,0 ng (middle quality control – MQC) and 4010,0 ng (upper quality control – UQC) in 1 ml of plasma of rabbits. Thus, we conducted a validation of the results obtained during the research. The analytical range of determination was from 8,02 up to 8020,0 ng in 1 ml of rabbit plasma.

The regulations of the pharmacokinetics studies.

To study the pharmacokinetics of SS-68 12 rabbits were pre-catheterized in the right ear vein so blood samples at all time points were taken from the same animals throughout the experiment. 12 hours before the start of the experiment the animals were deprived of feed, leaving free access to water. On the third day after catheterization were administered the test substance. Intravenous dosing the test substance was administered bolus of 6 rabbits in the ear vein in a solution of 22.0 mg/ml in water for injection at a dose of 2.2 mg/kg. Blood was sampled through a catheter in a volume of 0.3 ml in polypropylene tubes containing 20 μl of 5 % EDTA before using|, 5, 15, 30, 60, 120, 240, 480 and 1440 minutes after injection. When intragastric dosing of the substance is introduced using a probe in a solution of 22.0 mg/ml in water for injection at a dose of 22.0 mg/kg. Blood was sampled through a catheter in a volume of 0.3 ml in polypropylene tubes containing 20 μl of 5% EDTA prior to insertion, using 15, 30, 60, 120, 240, 480 and 1440 minutes after injection. Blood plasma was separated by centrifugation at 5600 g for 10 min and stored until analysis at -70 ºC.

To determine the concentration used validated the method of determining the SS-68 in the blood

plasma of rabbits in accordance with the Guidance on the examination of drugs under editorship of professor A. N. Mironov, volume I [16], as well as guidance for validation of bioanalytical methods FDA [17] and EMA [18], with the following characteristics: selectivity (specificity), linearity, accuracy and precision (intra-day and inter-day), quantification limit, robustness, the matrix effect and the carry-over.

Main pharmacokinetic parameters were calculated in accordance with the guidelines for preclinical studies of pharmaceuticals under the editorship of professor A. N. Mironov [19] in Microsoft Office Excel 2010 on the basis of the experimentally obtained data was calculated the pharmacokinetic parameters.

Dropping out results of the animals at each time point were detected by Grubbs statistical test [20]. This method showed good and accurate results [21, 22]. We calculated the arithmetic mean values and coefficient of variation (CV) for 6 animals.

Aaveraged pharmacokinetic curves and calculate the main pharmacokinetic parameters were constructed on the basis of the data obtained. Calculations made no model method, statistical analysis was performed in Excel.

Results and discussion.

Studies have shown that the SS-68 was rapidly absorbed from the gastrointestinal tract (mean time of absorption (MAT) - 36.3 min) and enters the systemic circulation within 15 minutes after administration. The maximum concentration (C_{max}) was 296.8 ng / mL of plasma. Then a rapid decrease of the concentration, the presence of characteristic plateau after 24 hours study approaching the threshold determination method. Reduced bore biexponential character, suggesting a rapid distribution of the first phase is replaced by a slower elimination phase. The presence of a plateau on the concentration-time curve shows several phases of distribution, which occur in sequence. Phase distribution and elimination for intravenous and intragastric administration at different times. This is due to the different time to achieve C_{max}. For two hours study SS-68 decreased the concentration of only 1.6 times (determined by the second hour 179,6 (CV – 17,6%) ng / ml plasma). This indicates that the SS-68 undergoes slow elimination in rabbits body. The averaged pharmacokinetic curves with a graphical display of the CV for each point shown in Figure 1.

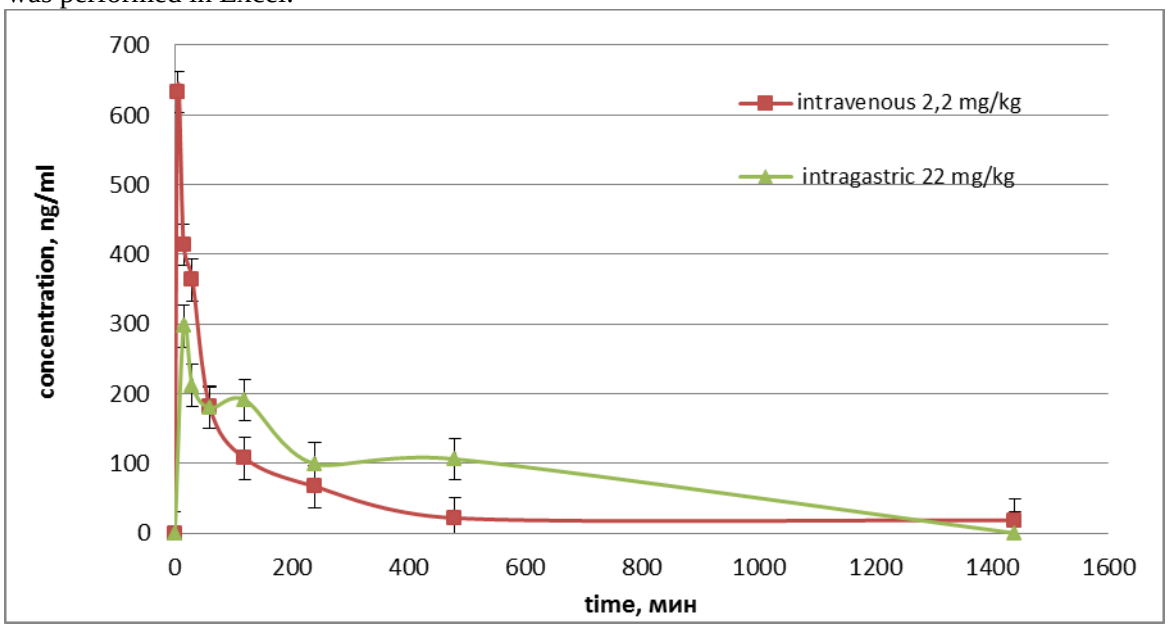

Figure 1. Mean plasma concentration-time curve of SS-68 (n = 6).

Main pharmacokinetic parameters (Table. 1) show the average half-life ($T_{1/2}$ = 2.5 h intragastrically and $T_{1/2}$ = 3.9 h intravenously) and the average retention time in the organism ($MRT_{(0-t)}$ = 5.5 h intragastrically, $MRT_{(0-t)}$ = 4.9 h intravenously). The areas under the concentration-time curve ($AUC_{(0-1440)}$ = 116,128.1 ng/mL×min in intragastrically, $AUC_{(0-1440)}$ = 69686.3 ng/mL×min intravenously). The magnitude of the stationary distribution volume (Vss) is equal to 10.8 l/ kg, far more than the extracellular fluid volume in the body of rabbits, indicating that high ability of the drug distributed and accumulate in tissues. Related to this low value indicator systemic clearance (Cl = 0.032 l/h).

The obtained pharmacokinetic parameters for different ways of doing showed that when administered intragastrically $T_{1/2}$ = 2.5 h (151.7 min)

which is lower than by intravenous administration $T_{1/2}$ = 3.9 hours (235.9 min). The absolute bioavailability (f_a) with intragastric administration was 16.6%. This fact is a certain rationale for the development of injectable form of the drug.

Table. 1

Mean pharmacokinetic parameters for SS-68 in the plasma rabbit (M±m; n = 6)

Pharmacokinetic parameters	Result	
	intragastrically	intravenously
$AUC_{(0-1440)}^{*}$, (ng/mil)×min	116128±34025	69686±3693
$AUMC_{(0-1440)}^{*}$, (ng/mil)×min ^{2}	37656266±10882661	23754516±3278123
$MRT^{*}_{(0-t)}$, min	328,5±10,8	292,2±33,606
Kel^{*}, min^{-1}	0,00490±0,00145	0,00297±0,00036
$T_{1/2}^{*}$, min	151,7±48,9	235,9±27,1
C_{max}^{*}, ng/ml	301,1±44,5	632,8±70,9
$C_{max\ iv}/AUC^{*}_{(0-1440)}$, min^{-1}	0,00270±0,00056	0,00907±0,00068
t_{max}^{*}, min	17,5±6,1	-
$C1_T^{*}$, l/h	0,032±0,002	-
V_{ss}^{*}, l/kg	10,7±1,2	-
f_a^{*}, %	16,6±4,5	-
MAT^{*}, min	36,3±10,3	-

*Note: $AUC_{(0-1440)}$ – the area under the concentration-time curve from 0 to the last sampling point (ng /ml)×min; $AUMC_{(0-1440)}$ – derivative of the area under the concentration-time curve from 0 to the last sampling point (ng /ml)×min^2; $MRT_{(0-t)}$ – mean residence time of the substance in the body, min; Kel – elimination constant, min^{-1}; $T_{1/2}$ – half-life, min; C_{max} – the maximum concentration, ng/ml; $C_{max\ iv}/AUC_{(0-1440)}$ – absorption speed, min^{-1}; t_{max} – time to maximum concentration of mines; $C1_T$ – systemic clearance, l / h; V_{ss} – steady volume of distribution, l / kg; fa – absolute bioavailability,%; MAT – C1т time, min.

Conclusions.

1. The studies of basic parameters SS-68 pharmacokinetics allowed to develop a method for the quantitative determination of the substance in the blood plasma of rabbits.

2. The pharmacokinetics SS-68 by intravenous and intragastric administration to rabbits. It is found that the drug is rapidly absorbed (MAT = 36,3 min) is well distributed in the tissues (Vss, = 10,8 l/kg) and has a $T_{1/2}$ of 3.9 h (235.9 min) after intravenous dosing. It has a low absolute bioavailability (fa = 16,6%), by the oral route.

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