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EXPERIMENTAL PHARMACOLOGY

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ANTICHOLINERGIC ACTIVITY AND PHARMACOKINETIC PARAMETERS OF AGENT SS-68 WITH PROPERTIES OF CLASS III ANTIARRHYTHMIC DRUGS

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Abstract

It was shown that the indole derivate SS-68 (50 and 250 µg/kg intravenous) in acute experiments on cats with neurogenic atrial fibrillation (AF) has a dose-dependent antiarrhythmic action which is associated with the neurotropic influence of this substance, since the suppression of the AF coincides with its anticholinergic effect observed for more than 2 hours, but cardiotropic action at this time was not observed. High antiarrhythmic activity of SS-68 to neurogenic AF may be due to the I_{KAch} inhibition and blockade of M_2-cholinergic receptors.

SS-68 (50 µg/kg intravenous) in experiments on rabbits is characterized by the following pharmacokinetic parameters: C_{max} = 14.5 ng/ml of blood plasma (duration of plasma exposure of SS-68 is 4 hours), $T_{1/2}$ = 1,1 ± 0.06 hours, $MRT_{(0-t)}$ = 1.6 ± 0.08 hours, $AUC_{(0-1440)}$ = 1006.1 ± 147.7 ng/ml×min, V_{ss} = 4.8 ± 0.8 l/kg and CL = 0.051 l/h.

Based on pharmacodynamic and pharmacokinetic parameters it was suggested that anticholinergic action of SS-68 in neurogenic AF will occur in a much lower dose than 50 mg/kg.

Key words: vagus nerve, neurogenic atrial fibrillation, indole derivate SS-68, antiarrhythmic action, pharmacokinetics.

Introduction

Atrial fibrillation is the most common heart beat disorder. For the 50-year period of observation in the Framingham study, the morbidity of AF corrected to age increased fourfold [1]. From 1990 to 2010, the prevalence of AF in the world and associated with it morbidity and mortality, despite all efforts of medical science and health care increased twofold [2]. According to forecasts over the next 3 decades the number of patients with AF in Europe and the United States will increase by more than twofold, which is reason enough to call this arrhythmia "global epidemic" [3, 4, 5].

In recent years in the treatment of AF there is the preference for class III antiarrhythmic drugs (classification by E. M. Vaughan-Williams [6]), acting mainly at the expense of slowing repolarization and increasing the duration of refractory periods [7].

Despite the fact that to the present time in our country and abroad there were created a number of different on the effectiveness and mechanism of

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action of class III antiarrhythmic drugs (amiodarone, sotalol, bretylium, dofetilide, ibutilide, azimilide, vernakalant, dronedarone, nibentan, niferidil), search and development of representatives of this group of drugs continues, that is due to its not enough effectiveness and presence of side effects, the main of which is the torsades de pointes [7].

Experimental study of effectiveness and mode of action of new class III antiarrhythmic drugs is carried out using various models of auricular fluttering [8, 9]. Of these, greater attention should be a model of neurogenic AF [8, 10], simulating in healthy animals without prior myocardial damage by steam stimulation of the atria on the back of vagal asystolism, initiated by irritation of the vagus nerve. This model in comparison with the known analogues, in which different approaches are used to development and maintenance of AF [8, 11, 12, 13, 14, 15], is not associated with artificial irregularities of the internal environment and is control that brings it the most to the pathogenesis of AF in clinical practice [16, 17]. Simulation of neurogenic AF made possible to study antifibrillatory action of many substances, including representatives of class III antiarrhythmic drugs [18, 19, 20, 21, 22, 23].

In previous studies it was shown that SS-68 on the mode of cardiotropic action may be fits into class III antiarrhythmic drugs [24]. It should be noted that in experiments on dogs SS-68 suppresses auricular fluttering [25], which is simulated by a mechanical destruction of the sinus node and subsequent electrical stimulation of the right atrium according to the method described by P. A. Galenko-Yaroshevskii et al. [8] A. Rosenblueth, G. Ramos [26]. Anticholinergic activity of SS-68 in the neurogenic AF has not been studied. In addition, the main pharmacokinetic parameters of the minimum effective doses of SS-68 have not been studied.

The aim of this study was to study anticholinergic activity of SS-68 in the neurogenic AF and identify its main pharmacokinetic parameters in minimum anticholinergic dose.

Experimental

Experiments to study anticholinergic activity of SS-68 in neurogenic AF were performed on 16 cats weighing 2.6 to 3.4 kg with anesthesia by intraperitoneal administration of 1% of a mixture of α-chloralose and aethaminalum-natrium (75 and 15 mg/kg, respectively) and artificial pulmonary ventilation according to the method developed by Y. R. Sheikh-Zade, P. A. Galenko-Yaroshevsky [10].

Neurogenic AF was simulated by application to the endocardium of the right atrium 2 electric pulses (5 ms, 4 threshold) with an interval of 40 ms on the back of rhythmic stimulation of the cervical portion of the right vagus nerve (2 ms, 40 Hz, 6 thresholds) using the electrostimulator ESU-2. ECG tracing (intra-atrial) was performed using beat-to-beat ratemeter on the recording meter H-338-2, while visual inspection of the events was conducted by using an 8-channel indicator IM 789. Agent SS-68 was administered intravenous in doses of 50 and 250 mg/kg.

In the beginning of the experiment, and then after the administration of the SS-68 at 5, 30, 60 and 120 minutes there were determined the duration of the intervals P-P and P-Q ECG, sinoatrial conduction time [27, 28], excitation thresholds of the atria and vagus nerve, atrial effective refractory period, the duration of AF, synchronizing and tonic components of the chronotropic effect of vagus nerve [8].

The research results were statistically analyzed. by the method of direct and indirect differences [29, 30] with the definition of arithmetic average (M), standard error of the mean (± m) and indices of statistical significance (p). The differences were considered significant at p < 0.05.

The pharmacokinetics of SS-68 was studied on 12 chinchilla rabbits, which were pre-catheterized in the right ear vein. 12 hours before the start of the experiment the animals were deprived of feed, leaving free access to water. The test substance was administered on the third day after catheterization intravenous bolus to 6 rabbits in the ear vein in the form of a solution of 500 μg/ml in water for injection at a dose of 50 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 administration and 5, 15, 30, 60, 120, 240, 480 and 1440 minutes after administration. Blood plasma was separated by centrifugation at 5600 g for 10 min and stored until analysis at -70 C.

To determine the concentration there was used previously validated method for determining SS-68 in the blood plasma of rabbits. Main pharmacokinetic parameters were calculated by A. A. Firsov et al. [31] in Microsoft Office Excel. Outliers in each time point were identified using a statistical test of Grubbs [32]. This test is well-proven in similar studies [33].

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Quantification of SS-68 in the blood plasma of rabbits was carried out on a liquid chromatograph LC UltiMate 3000 (Thermo Fisher Scientific, USA). Detection of analyte was performed on a mass spectrometer Velos Pro (Thermo Scientific, USA) with hot electrospray ionization (H-ESI-II). Chromatographic separation was performed on a column size 150×3.0 mm, filled by reversed-phase sorbent Zorbax Eclipce XDB C18 with particle size 3.0 mm with protective column Zorbax Eclipce XDB C18 12.5×3.0 mm with a particle size of 5.0 μm at a temperature of $40 \circ C$ in the mode of linear gradient of eluant at a flow rate of 0.4 ml/min by the following program:

Separation stage: Eluant A (5 mM Ammonium acetate + 0.1% formic acid) 55% → 45%; Eluant b (acetonitrile) 45% → 55% for 4 minutes;

Washing stage: Eluant A (5 mM Ammonium acetate + 0.1% formic acid) 45%; Eluant b (acetonitrile) – 55% – 0,5 minutes;

Trim stage: Eluant A (5 mM Ammonium acetate + 0.1% formic acid) – 55%; Eluant b (acetonitrile) 45% for 2 minutes.

The volume of injected sample was 5 μl. Scanning was performed by selected-ion monitoring (SIM). The transition mass for SS-68 – $305,26 \rightarrow 208,0$, internal standard (fabomotizole) – $307,41 \rightarrow 114,0$.

Results and evaluation

SS-68 at a dose of 50 mg/kg at 5, 30, 60 and 120 min after intravenous administration caused a statistically significant reduction in the duration of AF 85, 73, 50 and 33%, respectively. The intervals P-P and P-Q of ECG, the excitation threshold of the atrial effective refractory period and sinoatrial conduction of excitation were not be significantly changed. Against this background, SS-68 had vagolytic effect, manifested a significant increase in excitation thresholds of vagus nerve 16% (to the 5th min of the study), the synchronizing and tonic components of the chronotropic effect of vagus nerve, respectively, 50 and 47, 41 and 41, 41 and 37, 37 and 31% (to the 5, 30, 60 and 120 th min of the study) (table 1).

With increasing dose of SS-68 to 250 mg/kg at 5, 30, 60 and 120 min was a statistically significant reduction in the duration of AF 92, 84, 62 and 54%, respectively. Intervals P-P and P-Q of ECG, as well as the effective refractory period was significantly increased, there were P-P 13, 10 and 8% (5, 30 and 60th min), P-Q 22 and 14% (for 5 and 30 min) and effective refractory period 18, 13 and 12% (5, 30 and 60th min of the study). Excitation thresholds of the atria and sinoatrial conduction of excitation remained virtually unchanged. Vagolytic activity of SS-68 was manifested in the significant increase of the excitation thresholds of vagus nerve 24% and 14% (for 5 and 30 min) and the inhibition of the tonic and synchronizing components of the chronotropic effect of this nerve 62 and 58, 60 and 52, 44 and 58, 56 and 42%, respectively (to the 5, 30, 60 and 120 th min of the study) (table 2).

It is known that increased tone of the parasympathetic nerves causes a reduction in the duration of AF of cardiomyocytes of the atria due to the acetylcholine-activated outgoing K^+-current (I_{KAch}) [34, 35]. It is shown that many class III antiarrhythmic drugs (sotalol, E-4031, MS-551, amiodarone, ambasilide, nibentan, niterider) have a preventive and neutralize effect against cholinergic AF, which is due to the inhibition of I_{KAch} by blockade of M_2-cholinergic receptors associated with I_{KAch} by regulatory β – and γ-subunits of the G protein [34, 35], and/or directly I_{KAch} channel [36, 37, 38].

In recent experiments on mammalian cardiac myocytes (whole cell mode using the method of patch-clamp [8, 39, 40]) there was found that the greatest sensitivity to SS-68 has I_{KAch}, L-type Ca^{2+} current I_{CaL} and fast K^+-current of the detained straightening I_{Kr} (median effective concentration IC_{50} of SS-68 = 1.28, 1.78 and 2.75 μm, respectively) and moderate sensitivity characteristic for ultra-rapid delayed-rectifier K^+-current I_{Kur} and ATP-dependent inwardly rectifying K^+-current I_{KATP} (IC_{50} of SS-68 = 19.8 and 20.0 μm, respectively), low sensitivity to transient outward potassium current Ito (IC_{50} of SS-68 = 165.5 μm). Weakly susceptible to the action of SS-68 there were the fast Na^+ current I_{Na}, inward rectifying K^+-current I_{K1}, and slow component a current of the detained straightening IKs [24]. In addition, in experiments on preparations of the left atrium of the rat (with the registration of the action potential by the method of intracellular lead of bioelectrical activity [41]) there was shown that SS-68 at a concentration of 10 μm (definition of minimum effective concentration was not conducted) weakens the effects of general stimulation of the M_2 – and M_3-cholinergic receptors by pilocarpine (at concentration of 10 μm) [42]. Since the decline in

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SS-68 effect of stimulation of M_3-cholinergic receptors may not be related to its effect on I_{KAch}, because it is activated only by stimulation of M_2 and M_4 cholinergic receptors [43], the last of which in rat cardiomyocytes play a minor role, and M_1-cholinergic receptors are absent [44, 45, 46], we can assume that the antiarrhythmic effect of SS-68 on neurogenic AF is associated with its inhibitory effect on I_{KAch} and blocking M_2-cholinergic receptors.

By the definition of the main pharmacokinetic parameters of SS-68 at a dose of 50 µg/kg has been established that its maximum concentration in plasma (C_{max}) is 14.5 ng/ml; duration of content of SS-68 in the blood is 4 hours. The shape of the concentration time curve is biexponential, suggesting rapid first phase of distribution, alternating a slower phase of elimination. The presence of a plateau indicates a multi-phase distribution, which occur sequentially. Two hours of the study, the concentration of SS-68 reduced 7.2 times (the second hour there was determined 2.0 ng/ml in blood plasma). It suggests that SS-68 in such dose rapidly eliminates in rabbits. Main pharmacokinetic parameters did not show high values of elimination half-life ($T_{1/2}$ = 1.1 ± 0.06 hours) and the middle residence time ($MRT_{(0-t)}$ = 1.6 ± 0.08 hours). Fast reduction of concentration in plasma causes a small value of the area under the curve ($AUC_{(0-1440)}$ = 1006.1 ± 147.7 ng/ml × min. The value of the stationary distribution volume (V_{ss}) equal to 4.8+0.8 l/kg, that exceeds the amount of extracellular fluid in the body of rabbits, indicating a high ability of the drug to distribut and accumulate in the tissues. It is connected with the low value of systemic clearance (CL = 0.051 l/h).

Thus, SS-68 in conditions of neurogenic AF, caused by the irritation of the vagus nerve in cats, has a dose-dependent antiarrhythmic effect that is associated with the pre-emptive neurotropic influence of this agent, since the neutralize of the AF coincides with its anticholinergic effect observed over 2 hours and cardiotropic action at this time is absent. High antiarrhythmic activity of SS-68 in neurogenic AF may be due to the inhibition of I_{KAch} and blockade of M_2-cholinergic receptors. It is not excluded that antiarrhythmic activity of SS-68 in neurogenic AF will manifest itself in lower doses. This is shown by the pharmacodynamic and pharmacokinetic parameters of SS-68. To confirm this assumption more researches of the influence of SS-68 in neurogenic AF in the lower dose range are needed.

Table 1

The influence of SS-68 (50 µg/kg intravenous) on the cardiac output and the duration of AF when irritation of the vagus nerve in cats (M ± m, n = 8)

The indicators and their dimension	Source values	Duration of the action, min			
		5	30	60	120
Baseline duration of the P-P interval of ECG, msec	388.0 ± 14.0	376.0 ± 13.0 [97.0]	387.0 ± 14.0 [100.0]	384.0 ± 15.0 [99.0]	385.0 ± 15.0 [99.0]
Excitation threshold of atria, mV	438.0 ± 60.0	476.0 ± 90.0 [109.0]	458.0 ± 80.0 [105.0]	402.0 ± 70.0 [92.0]	415.0 ± 60.0 [95.0]
Effective refractory period of a myocard, msec	142.0 ± 8.0	138.0 ± 6.0 [97.0]	135.0 ± 8.0 [95.0]	140.0 ± 6.0 [100.0]	139.0 ± 5.0 [90.0]
Sinoatrial conduction of excitation. msec	28.0 ± 3.0	26.0 ± 2.0 [93.0]	29.0 ± 3.0 [104.0]	27.0 ± 2.0 [96.0]	25.0 ± 2.0 [89.0]
P-Q interval of ECG, msec	75.0 ± 4.0	73.0 ± 2.0 [97.0]	76.0 ± 3.0 [101.0]	74.0 ± 2.0 [99.0]	77.0 ± 4.0 [103.0]
Excitation thresholds of vagus nerve, mV	380.0 ± 40.0	440.0 ± 20.0* [116.0]	410.0 ± 50.0 [108.0]	400.0 ± 40.0 [105.0]	410.0 ± 50.0 [108.0]
Synchronizing component of the chronotropic effect of vagus nerve, msec	259.0 ± 28.0	130.0 ± 10.0* [50.0]	153.0 ± 24.0* [59.0]	154.0 ± 20.0* [59.0]	162.0 ± 25.0* [63.0]
Tonic component of the chronotropic effect of vagus nerve, msec	98.0 ± 12.0	52.0 ± 5.0* [53.0]	58.0 ± 12.0* [59.0]	62.0 ± 14.0* [63.0]	68.0 ± 10.0* [69.0]
Duration of AF, sec	140.0 ± 7.0	21.0 ± 4.0* [15.0]	38.0 ± 6.0* [27.0]	70.0 ± 12.0* [50.0]	94.0 ± 14.0* [67.0]

Comment: Here and in table 2: in brackets there are the indicators expressed in %; *$p < 0.05$ in comparison with baseline data.

Table 2

The influence of SS-68 (250 μg/kg intravenous) on the cardiac output and the duration of AF when irritation of the vagus nerve in cats (M ± m, n = 8)

The indicators and their dimension	Source values	Duration of the action, min			
		5	30	60	120
Baseline duration of the P-P interval of ECG, msec	352.0 ± 8.0	398.0 ± 9.0* [113]	387.0 ± 6.0* [110.0]	379.0 ± 6.0* [108.0]	360.0 ± 13 [102.0]
Excitation threshold of atria, mV	540.0 ± 60.0	560.0 ± 70.0* [117.0]	560.0 ± 80.0 [104.0]	530.0 ± 50.0 [98.0]	520.0 ± 60.0 [96.0]
Effective refractory period of a myocard, msec	136.0 ± 5.0	160.0 ± 4.0 [104.0]	150.0 ± 5.0 [102.0]	152.0 ± 4.0* [112.0]	144.0 ± 9.0 [106.0]
Sinoatrial conduction of excitation. msec	22.0 ± 2.0	22.0 ± 2.0 [100.0]	22.0 ± 2.0 [100.0]	21.0 ± 1.0 [95.0]	20.0 ± 2.0 [91.0]
P-Q interval of ECG, msec	72.0 ± 2.0	88.0 ± 2.0* [122.0]	82.0 ± 3.0* [114.0]	73.0 ± 3.0 [101.0]	70.0 ± 3.0 [97.0]
Excitation thresholds of vagus nerve, mV	370.0 ± 40.0	460.0 ± 30.0* [124.0]	420.0 ± 30.0* [114.0]	390.0 ± 40.0 [105.0]	380.0 ± 30.0 [103.0]
Synchronizing component of the chronotropic effect of vagus nerve, msec	265.0 ± 22.0	101.0 ± 28.0* [38.0]	106.0 ± 23.0* [40.0]	111.0 ± 22.0* [42.0]	117.0 ± 23.0* [44.0]
Tonic component of the chronotropic effect of vagus nerve, msec	94.0 ± 12.0	39.0 ± 6.0* [42.0]	45.0 ± 12.0* [48.0]	53.0 ± 14.0* [56.0]	55.0 ± 13.0* [58.0]
Duration of AF, sec	15.0 ± 8.0	12.0 ± 4.0* [8.0]	24.0 ± 5.0* [16.0]	58.0 ± 6.0* [38.0]	7.0. ± 6.0* [46.0]

References

1. Schnabel R.B., Yin X., Gona P. et al. 50-year trends in atrial-fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: A cohort study. *Lancet.* Vol. 386 (2015): 154-162. [PubMed]

2. Chugh S.S., Havmoeller R., Narayanan K. et al. Worldwide Epidemiology of Atrial Fibrillation: A Global Burden of Disease 2010 Study. *Circulation.* Vol. 129, № 8 (2014): 837-847. [PubMed]

3. Ball J., Carrington M.J., McMurray J.J.V., Stewart S. Atrial fibrillation: profile and burden of an evolving epidemic in the 21st century. *Int. J. Cardiol.* Vol. 167, №5 (2013): 1807-1824. [PubMed]

4. Reardon G., Nelson W.W., Patel A.A. et al. Prevalence of atrial fibrillation in US nursing homes: results from the national nursing home survey, 1985-2004. *J. Am. Med. Dir. Assoc.* Vol. 13 (2012): 529-534. [PubMed]

5. Wilke T., Groth A., Mueller S. et al. Incidence and prevalence of atrial fibrillation: an analysis based on 8.3 million patients. *Europace.* Vol. 15, №4 (2013): 486-493. [PubMed]

6. Vaughan-Williams E.M. Classification of antiarrhythmic drugs. *J. Cardiovasc. Pharmacol.* Suppl. 2 (1992): SI-S7.

7. Nesterenko L.Yu., Mironov N.Yu., Golitsyn S.P. Antiarrhythmic drugs. In book: The guide to cardiology in four volumes. Volume 4: cardiovascular Disease (II) / Under the editorship of Akademician E. I., Chazov. – M.: Practice, 2014. – P. 703-763. (In Russian)

8. Galenko-Yaroshevskii P.A., Kaverina N.V., Kamkin A.G., et al. Methodological recommendations on preclinical study of antiarrhythmic drugs. In book: The guidelines for preclinical studies of pharmaceuticals. Part one. – M.: Grif and K, 2012. – P. 385-416. (In Russian)

9. Fedorov V.V, Sharifov O. F., Rosenshtrauh L.V. et al. Mechanism of antiarrhythmic action of nibentan in an experimental model of vagotonic atrial fibrillation in dogs. *Cardiology*. Vol. 39, №3 (1999): 45-56. (In Russian) [eLIBRARY]

10. Sheikh-Zade Yu.R., Galenko-Yaroshevskii P.A. Controlled atrial fibrillation and a possible mechanism for its occurrence. *Bulletin of experimental biology*. Vol. 104, №9 (1987): 261-263. (In Russian)

11. Boyden P.A. Effects of pharmacologic agents on induced atrial flutter in dogs with right atrial enlargements. *J. Cardiovasc. Pharmacol.* № 87 (1986): 170-177. [PubMed]

12. Feld G.K., Venkatesh N., Singh B.N. Pharmacologic conversion and suppresion of experimental canine atrial flutter: differing effects of d-sotalol, quinidine, and lidocaine and significance of changes in refractoriness and conduction. *Circulation.* 74 (1986): 197-204. [PubMed]

13. Frame L.H., Page R.I., Hoffman B.F. Atrial reentry araound an anatomic barrier with a partially refractory excitable gap. A canine model of atrial flutter. *Circ. Res.* Vol. 58, №4 (1986): 495-511. [PubMed]

14. Schoels W., Yang H., Gough W.B., El-Sherif N. Circus movement atrial flutter in the canine sterile pericarditis model: differential effects of procainamide on the components of the reentrant pathway. *Circ. Res.* Vol. 65 (1991): 1565-1579. [PubMed]

15. Wu K.M., Hoffman B.F. Effect of procainamide and N-acetylprocainamide on atrial fibrillation: studies in vivo and in vitro. *Circulation.* Vol. 76 (1987): 1397-1408. [PubMed]

16. Coumel P. Autonomic influences in atrial tachyarrhythmias. J. *Cardiovasc. Electrophysiol.* Vol. 7 (1996): 999-1007.

17. Rosenshtraukh L.V., Fedorov V.V., Sharifov O.F. Cholinergic Atrial Fibrilation. Chapter 34. Cardiac Electrophysiology: from cell to bedside 4th edition Philadelphia WB Saunders Company. – 2004.

18. Cherednik I.L. Antiarrhythmic effect isoptin in neurogenic atrial fibrillation in cats. *Bulletin of Arrhythmology*. №8 (1998): 121. (In Russian) [eLIBRARY]

19. Cherednik I.L. Antiarrhythmic dynamics of cordarone in neurogenic atrial fibrillation in cats. XVII Congress of physiologists of Russia: Theses of reports. – Rostov-na-Donu, 1998. – P. 321. (In Russian)

20. Cherednik I. L., Sheikh-Zade, Yu. R. Antiarrhythmic activity of amiodarone in neurogenic atrial fibrillations. *Bulletin of experimental biology*. Vol. 127, №3 (1999): 307-310. (In Russian) [eLIBRARY]

21. Cherednik I.L., Sheikh-Zade Yu.R., Galenko-Yaroshevskii P.A. Antiarrhythmic effects of allapinin in neurogenic atrial fibrillation. *Bulletin of experimental biology.* Vol. 127, № 2 (1999): 174-176. (In Russian) [eLIBRARY]

22. Cherednik I.L., Sheikh-Zade Yu.R., Galenko-Yaroshevskii P.A., Muhambetaliev H.I. Influence of nibentan on neurogenic atrial fibrillation in cats. *Bulletin of experimental biology*. №2 (2011): 122-124. (In Russian)

23. Cherednik I.L., Sheikh-Zade Yu.R., Fedorova E.F., et al., Antiarrhythmic effects of obsidan in neurogenic atrial fibrillation. Man and medicine: Abstracts of IX Russian national Congress 8 – 12 April 2002 – M., 2002. – P. 720. (In Russian)

24. Bogus S.K., Abramochkin D.V., Galenko-Yaroshevsky P.A. et al. Effects of a new antiarrhythmic drug SS-68 on electrical activity in working atrial and ventricular myocardium of mouse and their ionic mechanisms. *J. Pharmacol. Sci.* Vol. 128, №4 (2015): 202-207. [PubMed]

25. Bogus, K.S., Galenko-Yaroshevskii P.A., Suzdalev C.F. Antiarrhythmic activity of the indole derivative of the SS-68 in ventricular and atrial forms of arrhythmia. *New technologies.* №4 (2012): 274-283. (In Russian) [eLIBRARY]

26. Rosenblueth A., Ramos G. Studies of flutter and of fibrillation. The influence of artificial obstacles on experimental auricular flutter. *Heart. J.* Vol. 33, № 5 (1947): 677-682. [PubMed]

27. Strauss H.G., Bigger J.T., Saroff A.L., Giardina E.-Y.V. Electrophysiologic evaluation of sinus node function in patients with sinus node dysfunction. *Circulation.* Vol. 53, №5 (1976): 763-776. [PubMed]

28. Strauss H.G., Saroff A.L., Bigger J.T., Giardina E.-Y.V. Premature atrial stimulation as a key to the understanding of sino-atrial conduction in man. *Circulation.* Vol. 47, №1 (1973): 86-93. [PubMed]

29. Glants S. Biomedical statistics: Translate from English. – M.: Practice, 1998. – 459 p. (In Russian)

30. Lakin G.F. Biometrics. – M.: Higher school, 1980. – 293 p. (In Russian)

31. Firsov A.A., Zherdev V.P., Portnoi, Yu.A., etc. Guidelines for preclinical studies of pharmacokinetics of drugs. In book: The guidelines for preclinical studies of pharmaceuticals. Under the editorship of Mironov, A. N., Bunatyan N. D., Vasiliev A. N. etc. Part one. M.: Grif and K, 2012. – P. 845-855. (In Russian)

32. Grubb's Test for Detecting Outliers. URL: http://graphpad.com/quickcalcs/Grubbs1.cfm

33. Buzov A.A., Kulikov A.L., Avtina T.V. et al. 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. *Research result: pharmacology and clinical pharmacology.* Vol. 2, №1 (2) (2016): 52-57. [eLIBRARY]

34. Kurachi Y. G protein regulation cardiac muscarinic potassium channel. *Amer. J. Physiol.* Vol. 269 (1995): C821-C830. [PubMed]

35. Yatani A., Codina J., Brown A.M., Birnbaumer L. Direct activation of mammalian atrial muscarinic potassium channels by GTP regulatory protein. *Gk. Science.* Vol. 235 (1987): 207-211. [PubMed]

36. Mori K., Hara Y., Saito T. et al. Anticholinergic effects of class III antiarrhytmic drugs in guinea pig atrial cells. *Circulation.* Vol. 91 (1995): 2834- 2843. [PubMed]

37. Watanabe Y., Hara Y., Tamagava M., Nakaya H. Inhibitory effect of amiodaron on the muscarinic acetylcholine receptor-opereted potassium current in guinea pig atrial cells. *J. Pharmacol. Exp. Ther.* Vol. 279, №2 (1996): 617-624. [PubMed]

38. Zaza A., Malfatto G., Schwartz P.J. Effects on atrial repolarization of the interaction between K^+ channel blockers and muscarinic receptor stimulation. *J. Pharmacol. Exp. Ther.* Vol. 27, №3 (1995): 1095-1104. [PubMed]

39. Abramochkin D.V., Kuzmin V.S., Rosenshtrauh L.V. Ion channels and currents in cardiac fibers are the basis of modern electrophysiology of the heart. In book: The guide to cardiology in four volumes. Volume 1: Physiology and pathophysiology of the cardiovascular system. Edited by Academician E. I. Chazov. – M.: Practice, 2014. – P. 39-83. (In Russian) [eLIBRARY]

40. Hamill O.P., Marty A., Neher E. et al. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* Vol. 391 (1981): 85-100. [PubMed]

41. Hoffman B.F., Cranefield P.F. Electrophysiology of the heart. New York: McGraw-Hill Book Company Inc 1960. – 297 p.

42. Bogus S.K., Abramochkin D.V., Suzdalev K.F., Galenko-Yaroshevskii P.A. Agent SS-68 inhibits the electrophysiological effects of stimulation of M_3-cholinergic receptors in the myocardium of the left atrium of the rat. *Kuban scientific medical Bulletin.* № 2 (157) (2016): 36-40. [eLIBRARY]

43. Dhein S., Van Koppen C.J., Brodde O. Muscarinic receptors in the mammalian heart. *Pharmacol. Res.* Vol. 44, № 3 (2001): 161-182. [PubMed]

44. Abramochkin D.V., Suris M.A., Borodinova A.A. et al. M3 cholinoreceptors: new mediator of acetylcholine action on myocardium. *Neurochem. J.* Vol. 2. №1-2 (2008): 90-94.

45. Abramochkin D.V., Tapilina S.V., Sukhova G.S. et al. Functional M3 cholinoreceptors are present in pacemaker and working myocardium of murine heart. *Pflugers Arch.* Vol. 464. №4 (2012): 523-529. [Abstract]

46. Wang H., Shi H., Lu Y. et al. Pilocarpine modulates the cellular electrical properties of mammalian hearts by activating a cardiac M_3 receptor and a K^+ current. Br. *J. Pharmacol.* Vol. 126 (1999): 1725-1734. [PubMed]