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Original Article

PHARMACOKINETIC STUDIES OF NEW SILVER-BASED COMPLEX

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ABSTRACT

Objective: Investigation of pharmacokinetics parameters of the new silver-based compound of general formula $C_6H_{19}Ag_2N_4LiO_6S_2$, called "Argosil", with high antiviral activity.

Methods: Water solution of complex administered intragastrically in the dose rate of 100 mg/kg body weight of mice BALB/c (males) aged 1.5-2.0 mo. Blood and tissues samples analyzed by mass spectrometry.

Results: Half-life of the drug was about 4 h at the initial stage. About 5% of the initially introduced silver detected after 24 h. Maximum drug concentration observed in the lung tissues.

Conclusion: Results make the basis for further test and clinical implementation. Taken into account low toxicity and antiviral properties of this compound, it looks especially effective for lung infections treatment.

Keywords: Silver-based complex, Pharmacokinetics, Antiviral drug, Silver distribution in tissues, Mass spectrometry, Argosil.

INTRODUCTION

Since ancient times, silver has being used as anti-infective agent in various forms and applications. However, silver (nanoparticles, ionized form etc.) has significant toxic side effects, including argyria (deposition of insoluble silver in skin and cornea) [1]. An important challenge for a modern pharmacy is to create and study of new compounds contribute to solving the problem of infectious diseases and drug resistance. In this regard, it is topical to develop new silver compounds with high biological activity and low toxicity and the study of its pharmacokinetics parameters [2].

The purpose of this work-the study of the pharmacokinetics of the new silver-based compound of general formula $C_6H_{19}Ag_2N_4LiO_6S_2$, called "Argosil".

Previously, we have shown low toxicity and antiviral properties of compounds of this group [3].

MATERIALS AND METHODS

The object of study

The substance "Argosil" was tested as a 1% solution in double distilled autoclaved water with moderate heating (37 °C). As it previously established, 50% of the effective antiviral dose, ED_{50} equal 2.66 mg/kg. It has also been shown that the antimicrobial effect is achieved at doses approximately 100 times higher than the doses at which begins to show antiviral activity. Based on these data, dose of 100 mg/kg was chosen for present pharmacokinetics study. This dose reveal an antibacterial effect and significantly higher than the dose at which an antiviral effect is observed, so it covers all anti-infection spectrum.

Experimental animals

Mice BALB/c (males, 1.5-2.0 mo age) were used as a model animal for studying pharmacokinetics parameters. Six mice were used for each time point. Substance was administered intragastrically in water solution in the dose of 100 mg/kg body weight. Spleen, kidney, stomach, liver, lung, muscle, skin, blood from retro orbital sinus was selected for histological examination. Organs and tissue, sampling was performed at 5 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 16 h and 24 h after drug administration. The volume of the collected blood was not less than 1.0 ml. Biopsies of soft tissues (spleen, stomach, liver, kidneys, lungs, muscles) immediately after collection, preparation, removal of blood, weighed to the nearest 1 mg and placed in a plastic laboratory ware. Mass of tissue sample varied of 0.1 g (minimum) to 5 g. All the samples were stored at-18 °C, avoiding thawing tissues.

Sample preparation

The tissue samples were transferred into autoclave DAP 60K volume of 60 ml. Eppendorf tube was washed thoroughly twice 1.0-1.5 ml of concentrated nitric acid, after that washings joined to the sample. Mineralization of samples was carried out by adding 5-10 ml of concentrated nitric acid in an autoclave DAP 60K with microwave heating using a microwave sample preparation system-speed wave® MWS-2 (Berghof, Germany) according to the standard technique. After cooling, the clear solution purified from oxides of nitrogen and quantitatively transferred into a glass flask of 25 ml.

Analysis of samples

Sample analysis was performed by mass spectrometry with inductively coupled plasma (ICP-MS), using the quadrupole mass spectrometer 7500a Agilent (Agilent Technologies, USA).

The analysis performed according to the technical instruction manual of the device [1], using FullQuant mode with isotope $_{107}Ag$, three lines on the peak, and five replicates of measurements (fig. 1). Calibration curve traced by five calibration solutions prepared by diluting the standard solution (GSO 8204-2002).

Reagents

Deionized water was prepared using a water purification system Direct-Q 3 UV (Millipore, France) according to the technical manual. Resistivity of purified water was 18.2 mega-ohm (at 25 °C). Deionized water used to prepare working solutions, washing and rinsing autoclaves and volumetric glassware. The concentration of silver in the deionized water was less than 0.093 μg/l. Nitric acid of high purity. Commercially available concentrated nitric acid (standard sample GOST 4461-77) purified by double distillation (at a temperature below the boiling point) on the device Duo Pure (Milestone, Italy). The concentration of nitric acid in the distilled azeotropic mixture was 69.2%. The required solutions of nitric acid of high purity were prepared by diluting appropriate aliquots of concentrated acid with deionized water. Standard solution of silver for the calibration curve was prepared by diluting 0.3% HNO_3 with appropriate aliquots of the standard sample solution of silver ions (standard sample GSO 8204-2002 certified), manufactured by JSC "Ural Plant of Chemical Reagents". A solution of the blank sample is

prepared by adding aliquots of an acid sample without adding the sample after microwave heating; the transparent substrate transferred into a volumetric flask and diluted with 25 ml of deionized water to account for baseline concentrations.

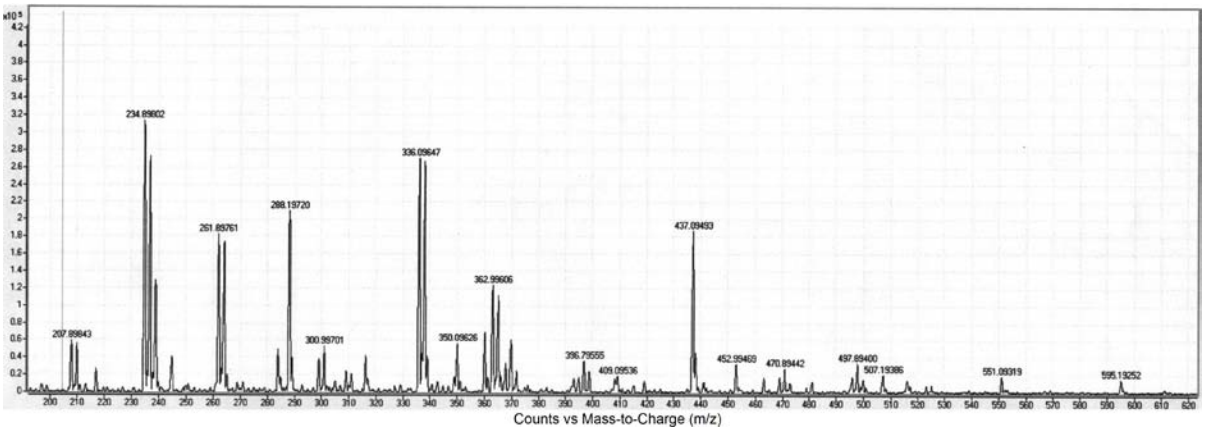

Fig. 1: Mass-spectrogram of "Argosil" sample

Processing of measurement results

The control program of spectrometer Chemstation [4] performed calibration curves and the calculation of the concentration of the element. Data processing performed by statistical software "Statgraphics, Vers.5.0" (Statistical Graphics Corp., USA). The accuracy of the detected intergroup differences were evaluated using Student t-test. Differences were significant at $p \leq 0.05$. Experimental data were checked against the normal distribution to assess the possibility of using the t-test. Pairwise comparison of samples performed using t-test. The results recalculated into weight of the complex based on the fact that silver is 37.2 mass percentage of complex "Argosil". Pharmacokinetic parameters were calculated in accordance with [5, 6].

RESULTS AND DISCUSSION

Specific drug content in the tissues and organs after a single intragastric administration of 100 mg/kg shown in table 1. Distribution is uneven because of differences in blood perfusion and tissue properties.

Table 1: The specific drug content in the mice tissues after a single administration

Time, h	Organs and tissues						
	Stomach, µg/g	Lungs, µg/g	Liver, µg/g	Spleen, µg/g	Kidney, µg/g	Blood, µg/g	Muscle, µg/g
0.08	374.4±9.7	0.129±0.02	1.320±0.46	0.146±0.03	3.72±0.29	0.972±0.10	3.39±0.26
0.5	321.9±3.9	16.79±1.05	2.489±0.43	1.728±0.18	2.67±0.36	1.303±0.29	3.03±0.55
1	257.0±4.2	19.98±2.29	2.49±0.11	4.31±0.48	1.28±0.18	2.32±0.33	6.35±0.67
2	175.4±7.1	19.85±0.79	2.44±0.43	4.97±1.57	1.54±0.36	3.314±0.09	4.76±1.43
4	117.6±3.5	29.85±0.79	5.086±0.40	5.104±0.74	6.67±1.72	4.093±0.72	4.10±0.59
6	92.00±3.1	20.68±3.47	10.52±1.47	8.25±0.70	5.35±0.39	3.55±0.64	3.15±0.48
8	67.39±3.1	17.39±2.04	14.17±1.58	14.24±1.07	7.62±0.91	2.81±0.37	4.37±0.59
10	45.05±2.67	11.28±0.90	13.65±1.56	13.30±1.32	5.34±0.83	1.63±0.21	1.95±0.48
16	23.40±1.04	6.24±0.73	7.99±1.20	10.07±0.93	3.80±0.68	0.68±0.05	0.83±0.38
24	7.99±0.55	1.79±0.65	3.99±1.29	4.63±0.61	0.64±0.07	0.41±0.04	0.75±0.15

Percentage of the drug found in the organs of mice at different time points after drug administration, shown in fig. 2. The 100% point is total content of the drug, revealed in all samples after 5 min of administration. In the initial phase, half-life of the drug was about 3 h. After 24 h, in the body is detected approximately 5% of the initially introduced silver (fig. 2). After 48 h revealed about 2% of the total silver amount, detected in the first 5 min after its administration.

Accumulation and excretion of the drug from the tissue adequately approximated by the equation describing the model [5]: $C(t) = A_1 \times e^{-\alpha t} + A_2 \times e^{-\beta t}$,

where C (t)-the concentration of drug in the tissue at time t, µg/g; A_1 and A_2-the amount of drug which is eliminated from 1 g of tissue, respectively, the first and second phases of the process of elimination, µg/g; α and β-complex parameters, which are proportional to a constant rate of excretion of the drug in the first and second stages of the process.

Small amount of the drug (about 0.2% of the initially administered drug) appeared in the blood in 5 min after administration.

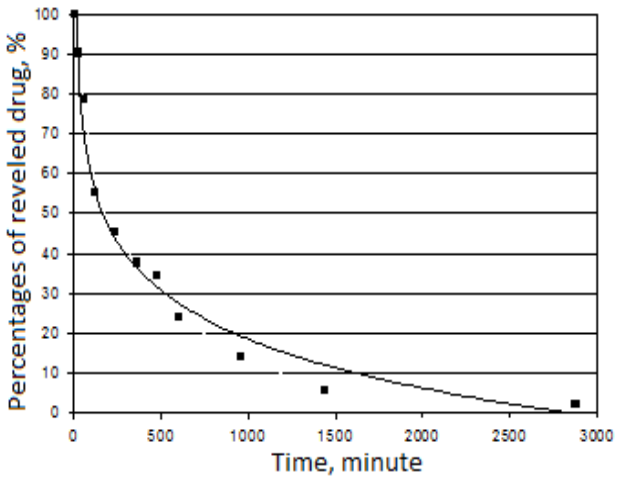

Fig. 2: The total content of the preparation in all samples

Fluctuations in the concentration range of 0.2-1.0% observed throughout the experiment. The maximum concentration (C_{max} = 4.093 g/ml) in the blood was observed after 4 h (t_{max}) drug administration. Pharmacokinetics parameters of the drug "Argosil" in tissues and organs of mice are given in table 2, where
AUC-area under the curve "concentration-time";

MRT-mean residence time of molecules of pharmacological agents in the body;

f_T-fraction of total amount of drug in the tissues-characterizes the intensity of penetration of pharmacological agents in the peripheral tissues

Table 2: Pharmacokinetic parameters of the drug "Argosil" in tissues and organs

Organ	AUC, (µg/g) min	MRT		Tissue fraction, f_T
		minute	hour	
Blood	3032	753	12.6	-
Stomach	104240	444	7.4	34.38
Lungs	17057	457	7.6	5.63
Spleen	1267	783	13.1	0.49
Liver	1204	766	12.8	0.40
Kidneys	531	683	11.4	0.18
Muscle	92	371	6.2	0.03

After 4 h, the drug concentration in the blood gradually decreases and its accumulation observed in other internal organs (spleen, liver, kidney t_{max} = 8 h; C_{max} = 14.22; 14.16; 7.60 µg/g, respectively). Only lungs are exceptions, in this organ the drug reaches maximum concentration after 4 h and C_{max} is 29.85 µg/g.

Analysis of muscle and skin tissues revealed no significant accumulation of silver in the course of the experiment.

CONCLUSION

It was shown maximum drug content observed in the stomach tissue at the intragastric administration. Accumulation and excretion of the drug "Argosil" from the tissue adequately approximated by the equation describing the model. Half-life of the drug was about 4 h at the initial stage. Taking into account the total content of the drug in all investigated organs and tissues, we may conclude rapid removal of the drug through the gastrointestinal tract. Accumulation of the drug in the internal organs have expressed time maximums (lungs-4 h, other organs-8 h). Maximum drug concentration observed also in the lung tissues. Lungs tissue fraction f_T is 5.63, which substantially exceeds the value f_T of other internal organs. In regards of this fact, and taken into account antiviral properties of this compound, "Argosil" can be more effective for lung infections treatment. The obtained results are an essential basis for further study of the mechanism of action of a new preparation and conduct of clinical trials.

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CONFLICT OF INTERESTS

Declared None

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