RESEARCH RESULT
НАУЧНЫЙ РЕЗУЛЬТАТ
Larin S.L., Zvyagintseva A.R., Habarov A.A., Budko E.V. Experimental investigation of pharmacokinetic properties and the accumulation of zinc when administrated nanoform of zinc hydroxide in a comparative aspect with zinc sulfate. Research result: pharmacology and clinical pharmacology. Vol. 2, №4 (2016): 21-33.

UDC: 615.015.45

DOI:10.18413/2500-235X-2016-2-4-21-33

Larin S.L.[1]
Zvyagintseva A.R.[2]
Habarov A.A.[3]
Budko E.V.[4]

EXPERIMENTAL INVESTIGATION OF PHARMACOKINETIC PROPERTIES AND THE ACCUMULATION OF ZINC WHEN ADMINISTRATED NANOFORM OF ZINC HYDROXIDE IN A COMPARATIVE ASPECT WITH ZINC SULFATE

1) Graduate student of the Department of General and Bioorganic Chemistry branch of Kursk State Medical University. 305041, Russia, Kursk, Karl Marx St., 3. E-mail: *sergeilarin.kursk@gmail.com*
2) Graduate student of the Department of Operative surgery and topographic anatomy branch of Kursk State Medical University 305041, Russia, Kursk, Karl Marx St., 3. E-mail: *annushka15zv@gmail.com*
3) Doctor of Pharmaceutical Sciences, and Professor of the Department of General and Bioorganic Chemistry of Kursk State Medical University. 305041, Russia, Kursk, Karl Marx St., 3. E-mail: *khabarov2014@mail.ru*
4) Doctor of Pharmaceutical Sciences, Professor and Head of the Department of General and Bioorganic Chemistry of Kursk State Medical University. 305041, Russia, Kursk, Karl Marx St., 3. E-mail: *e.v.ksmu@mail.ru*

Abstract. Pharmacological correction of the imbalance of zinc is important for a comprehensive treatment of microelementoses human. Existing zinc preparations limited in their application, so as active ingredients used oxide and zinc sulfate. Nowadays, with the development of physical and chemical methods of dispersion increases the amount of information about more effective action nanoforms zinc compounds. Via sol-gel method, nanoparticles (2-3 nm) of zinc hydroxide were obtained, which were measured by small angle X-ray scattering. The purpose of a preliminary assessment of the biological effect, a comparative study of nanoparticles using test cultures of bakery yeast, the results of which determined the level of inhibition, which was higher than 90%, compared with the figure for the zinc sulfate and the above 145% more compared to the zinc oxide. Investigation was performed on the pharmacokinetic characteristics of the 40 rabbits-males in comparison with that using zinc sulfate, in an enteral or intravenous administration at three dose levels: 10, 50 and 100 mg/kg. The time to reach maximum concentration of Zn^{2+} in enteral administration of nanoform was 4 hours, which is faster than that of zinc sulfate - 6 hours. The total clearance of nanoparticles increases with dose increases, amounting to 9.60±0.38, 13.96±0.24, 15.55±0.12 ml/h, half-life is not a statistically significant change. High parameters determined for the absolute bioavailability of zinc in enteral administration of zinc hydroxide nanoparticles - 30.33 ± 3.16, 44.39 ± 4.52 and 42.47 ± 3.66%, respectively, for doses of 10, 50 and 100 mg/kg. The pharmacokinetic properties when administered nanoforms zinc hydroxide and zinc sulfate soluble have no significant difference (at 10 and 50 mg/kg), which, however, are shown at a dose of 100 mg/kg. Determined a more rapid time of onset of maximum concentration for nanoforms, in comparison with zinc sulfate, and high parameters absolute bioavailability for zinc in enteral introduction of nanoparticles of zinc hydroxide: - 30.33±3.16, 44.39±4.52 and 42.47±3.66%, respectively, with a dosage: 10, 50 and 100 mg/kg. The pharmacokinetic properties when administered nanoform of zinc hydroxide and soluble zinc sulfate have no significant difference (at 10 and 50 mg/kg), which, however, have difference at 100 mg/kg. Investigation accumulation dynamics was conducted on 90 rats-males Wistar under enteral administration in a dosage of 100 mg/kg, using as comparison compound, zinc sulfate. Significantly was selected ($p<0.05$) the maximum level of accumulation of zinc in erythrocyte mass with the introduction of nanoforms of zinc hydroxide. Also significantly was selected ($p<0.05$) the lowest level of accumulation of zinc in liver tissues, compared with those groups which were administered zinc sulfate and zinc hydroxide.

Keywords: zinc, nanoparticles, zinc hydroxide, zinc sulfate, zinc oxide, pharmacokinetics, accumulation.

Introduction.

Our days more than 6% of all deaths in the world have a correlation with the incidence of microelementoses [1]. Zinc deficiency on the prevalence of inferior iron deficiency. By 2010, the figure connected with zinc deficiency mortality rate was 9136 people, with the total number exposed to geparinaza people in the world amounted to 1.1 billion people. [2]. It is important to note that zinc deficiency in the human body occurs not as a consequence of insufficient supply of food, and due to the low bioavailability from dietary sources. In addition, zinc deficiency can occur in severe diseases: sickle-cell anemia, AIDS, burns, liver cirrhosis, and others.

Some clinical condition associated with zinc deficiency, cover the skin, gastrointestinal tract, central nervous system, skeletal system and reproductive system [3]. Zinc deficiency manifests in weight loss, deterioration in the perception of taste and smell. Extensive influence on the function of the body is due to the structural and catalytic role of Zn^{2+} to more than 3000 enzymes. [4].

To correct the imbalance advisable to use zinc supplements [5]. Zinc oxide and sulfate zinc are the basic compounds that are part of the most vitamin-mineral complexes [6, 7]. Search of ways to increase therapeutic effect is divided in two areas: the traditional combination of zinc compounds to enhance the effect, and the search for new biologically active compounds among the products of modern technology [8]. A promising direction could be the application of ground to nanoscale (10^{-9} m) zinc compounds. It is known that significantly modifies the biological properties of the compound change in its physico-chemical properties: size, surface morphology and crystal structure [9]. The results of pharmacological and biopharmaceutical research conducted on laboratory animals, conducted for nanoparticles of zinc oxide [10, 11, 12, 13] and demonstrate a higher bioavailability parameters, compared with the traditional compounds zinc sulfate and zinc oxide.

Thus, the urgent problems of modern clinical pharmacology are: study of the interaction between the organism and zinc compounds dispersed to nanoscale, the study of their pharmacokinetics, the study of the distribution between organs and tissues, establishing relationships between dose, concentrations and efficiency.

The purpose of this investigation was to study the pharmacokinetic properties and bioavailability of nanoforms of zinc hydroxide and its distribution among tissues and organs in comparison with zinc sulfate in the experiment *in vivo*.

Materials and methods.

Synthesis of nanoparticles was performed zinc hydroxide sol-gel method in an environment of absolute ethanol (99.95%). Absolute ethanol was used to prepare solutions of zinc acetate dihydrate (chemically pure) and lithium hydroxide anhydrous. The solutions were merged with the cooling to 0°C at a speed of 2-3 drops per minute. The resulting suspension was diluted with distilled water and centrifuged to separate the gel that contains nanoparticles of zinc hydroxide. The particle size was estimated by the method of small angle x-ray scattering using an energy dispersive x-ray fluorescence spectrometer Shimadzu EDX-800HS.

Received nanoform of zinc hydroxide was subjected to a preliminary investigation *in vitro* on the test culture of *Saccharomyces cerevisiae*. The yeast activity was determined by accelerated method of determining the lift force on the State standard of the Russian Federation 54731-2011. The average samples of bakery compressed yeast was selected suspension of yeast with a mass of 0.31 g, which was transferred to a porcelain cup with the addition of 2.5% salt solution, with a volume of 4.8 cm^3 heated to 35°C. After thorough added with stirring, 7 grams of flour for the dough, which indulged in ball shape. This ball of dough dropped in a glass of water and thermostating at a temperature of 35°C. The lifting force was characterized by a period of time until the ascent of the ball, which was calculated in minutes and multiplied by an empirical coefficient 3.5. The lifting force of the control group (no added substances) was calculated as 100% and changes were calculated relative to 100%. Increasing the value shows a negative effect (increase in time of ascent of the ball of dough), with a decreasing value shows a negative effect (reducing the time of ascent of the ball of dough). For comparison of the effect were used: zinc oxide (commercial sample), zinc sulfate (soluble crystals) and zinc hydroxide (nanoparticles obtained by the sol-gel method).

Experiments using laboratory animals was planned and conducted in accordance with the Directive 2010/63/EU the European Parliament and of the Council of the European Union for the protection of animals used for scientific purposes, 22 September 2010, Council's Directive 86/609/EEC, 24 November 1986: "On the coordination of laws,

regulations and administrative orders of the participating countries regarding the protection of animals used for experimental and scientific purposes". The animals were kept indoors in individual cages with maintaining the specified parameters of the microclimate: temperature 20°C ± 2°C, humidity 60% ± 10% and 12-hour lighting cycle. Animals received a standard diet of the vivarium: extruded complete feed PK-120 and filtered drinking water in a quantity *ad libitum*. Animals were placed on 14-day quarantine before the manipulation, during which conducted monitoring of the clinical condition with daily visual inspection. 12 hours before the start of the experiment the experimental animals were deprived of food. Operations and manipulations with animals were performed using General anesthesia with intra-abdominal introduction of an aqueous solution of chloral hydrate at a dosage of 300 mg/kg. The killing was carried out by means overdose of anesthetic.

Experimental study of the pharmacokinetic profile of nanoscale zinc hydroxide was carried out on 40 rabbits-males of Chinchilla breed of Federal state unitary enterprise "Nursery of laboratory animals Rappula". Animals were selected according to body weight in the range from 4200 to 4430 grams.

The study of pharmacokinetic characteristics of zinc in a one-time enteral introduction of nanoforms of zinc hydroxide was carried out on 10 rabbits, which by the criterion of the amount of the drug was divided into 4 groups (n=10). Animals using the probe was introduced a suspension of nanoparticles of zinc hydroxide (in distilled water) in three doses: 10, 50 and 100 mg/kg (calculated as zinc), respectively, in the 1, 2 and 3 group. In the control group 4 rabbits were intragastrically administered an equivalent volume of distilled water. Similarly, to determine the pharmacokinetic properties of the compounds-comparison of the sulfate of zinc when enteral administered in the same dosage was formed 4 groups (n=10).

To determine the parameters of absolute bioavailability for nanoforms of zinc hydroxide and compounds-comparison of the zinc sulfate a study was conducted pharmacokinetic properties under conditions of intravenous administration at three doses: 10, 50 and 100 mg/kg. Nanoparticles were introduced as suspension in isotonic solution of sodium chloride. Zinc sulfate was administered as an aqueous solution for injection.

Sampling of blood with a volume of 1 ml was performed through a catheter from a regional ear vein every 15, 30, 60, 120, 240, 360, 480, 600, 720, 960 and 1440 minutes. Also the sampling of blood with a volume of 1 ml was carried out after intravenous injection every 1, 10, 15, 30, 60, 90, 120, 240, 360 and 720 minutes. Samples in test tubes that were treated with lithium heparin, placed in a centrifuge at 3000 rpm for 15 minutes to separate plasma. Before analysis the plasma was frozen and stored at a temperature of -28°C.

Investigation of the distribution in organs and tissues was conducted on 90 " rats-males breed Wistar aged 10 weeks and weighing in the range of 120 – 150 grams. Rats by the criterion of the obtained compounds were divided into 4 groups: nanoform of zinc hydroxide (group 1), microparticles of zinc oxide (group 2), the comparison compound of the zinc sulfate (group 3) and control group (group 4). Compounds were administered as a suspension (zinc hydroxide and zinc oxide) enteral method and in the form of a solution (zinc sulfate) at a dosage of 100 mg/kg (calculated as zinc). Animals received the test compound once a day under the scheme: 0 – 24 – 48 – 72 – 120 – 168 hours. Through 4 hours after the administration in an animal specimen using ether anesthesia blood sample was obtained from the jugular vein. After sacrifice of the animal were sampled testes and liver.

Pre sample preparation for the quantification of zinc in biological material was carried out by means of mineralization in perchloric acid (72.4%) at a temperature 190-210°C with oxidation by hydrogen peroxide (36%). Transparent mineralized evaporated to a state of moist salts and subsequently dissolved in deionized water and analyzed by atomic absorption spectrometry on the spectrometer "SPECTR-5-4" (JSC "Soyuztsvetmetavtomatika" number in the state register of measuring instruments 13743-04). Before the work was carried out the calibration method of absolute calibration with using of state standard samples of substance of zinc ions (LLC "TSSOVV", Russia, State registration number in the registry 8053-94) with a concentration in the range of 0.0005 – 1.0 mg/dm^3.

To obtain the characteristics of statistical indicators were calculated: the arithmetic mean number, standard deviation arithmetic mean number (Sd), standard error of the arithmetic mean of the number (m), coefficient of variation (CV%). To determine the statistical significance of differences was used the method of defining the boundaries of the confidence interval (t) with an acceptable level of $p<0.05$ for experimental biomedical research.

Mathematical calculations and plotting was done using the software OriginPro 9.2 (OriginLab, USA) and Excel 14 (Microsoft, USA).

Obtained results and their discussion.

The weight distribution function of inhomogeneity's in the Dm (d) was calculated along the curves of small angular scattering and demonstrates showed a predominance of particles of zinc hydroxide with a size of 2-3 nm (Fig. 1)

.

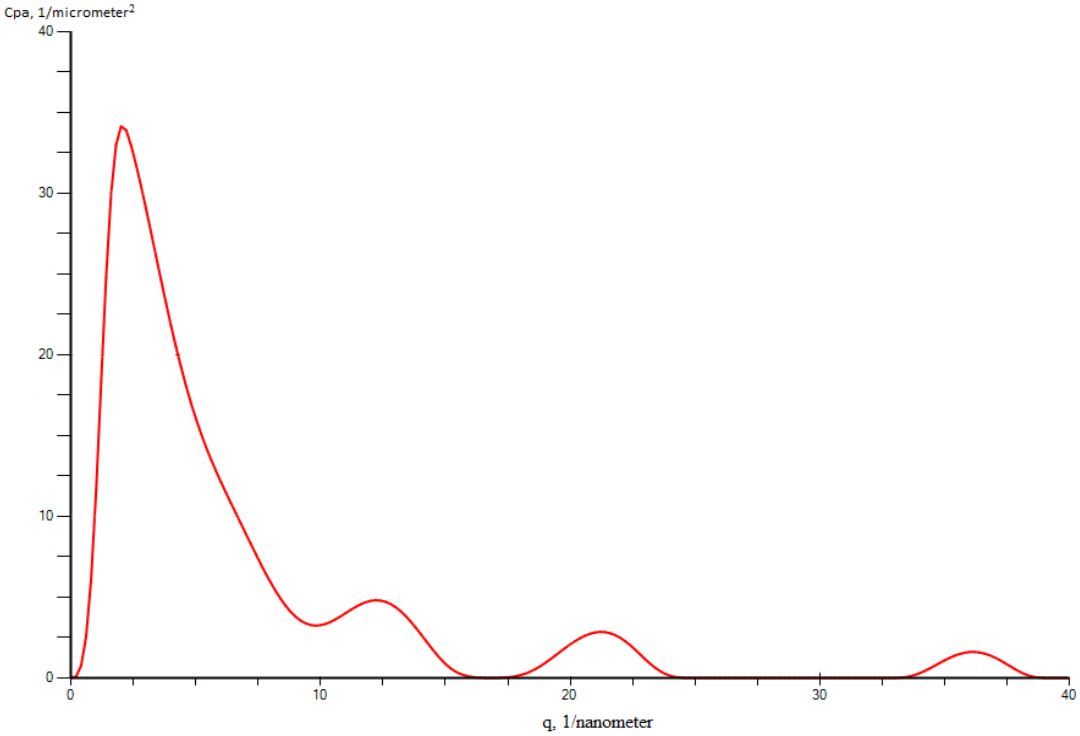

Figure 1. The distribution of inhomogeneity's in the gel containing nanoparticles of zinc hydroxide.

For a preliminary assessment of biological activity was used a test system from cell cultures *S.cerevisiae.* The system allows simulating the basic conditions of membrane transport, with the possibility of estimating efficiency without the use of special methods. Indicator of biological activity was the parameter of the lifting force of the yeast, because the fermentation process involved enzymes, localized in different parts of the cell.

Figure 2 shows the obtained dependence of the degree of change of the lifting force of the test culture *S.cerevisiae* from the content of the compound (calculated as zinc) on 1 gram of yeast cells at different concentrations.

Maximum depressing activity in the lift force was observed in the group (figure 2 (1)) corresponding to the nanoparticles of zinc hydroxide. Maximum observed at 48.39 mg/g and constitutes 272.83 ± 2.43%. The rate of lift force takes the value characteristic of "idle" experience with 3 mg/g. Zinc sulfate (figure 2 (2)) exerts a small inhibitory effect with increased 144.00±2.48% at 48.39 mg/g and does not have statistical differences compared with the control group at 12.1 mg/g. The rate of changing lifting force ($p < 0.05$) higher for the group, which was introduced nanoform of zinc hydroxide than for the group, which was administered zinc sulfate at concentrations of 48.39, 24.19 and 12.10 mg/g. An intermediate position is occupied by a group in which a commercial sample of zinc oxide was added to the test cultures (Figure 2 (3)), resulting in the inhibition effect was maintained at a concentration of 12.1 mg/g. Values lift changes were statistically indistinguishable with zinc sulfate group, since dosages 24.19 mg/g.

Several authors noted the need for the presence of a constant concentration of Zn^{2+} in the culture medium during the cultivation *S.cerevisiae* – 10-15 mM. This is necessary to maintain enzymatic activity [14] and can decrease the lift force (positive effect). The excess of zinc can have toxic influence on the culture of yeast, decreasing membrane permeability for ions K^+ and inhibiting the activity of glycosidase, increasing the value of the lift force. [15]. Formation mechanism of inhibitory action of nanoparticles of zinc hydroxide based on the formation of reactive oxygen species (ROS) within the cell. [16, 17]. The main toxic effect is

on the cell membrane, where localized basic proteins during alcoholic fermentation. However, the original mechanism of damage is formed in the mitochondria, which are transported in the nanoparticles absorbed by endocytosis. Membrane damage occurs after damage to the walls of the mitochondria and after penetration of ROS into the cytosol. This inevitably leads to the immobilization of enzymes and to increase the lift. The increase of the lift force is an inevitable process when activating transport nanoforms through the cell wall. This allows making a conclusion about the nature of the inhibitory action of zinc hydroxide due to its complete penetration through the membrane of the yeast cells.

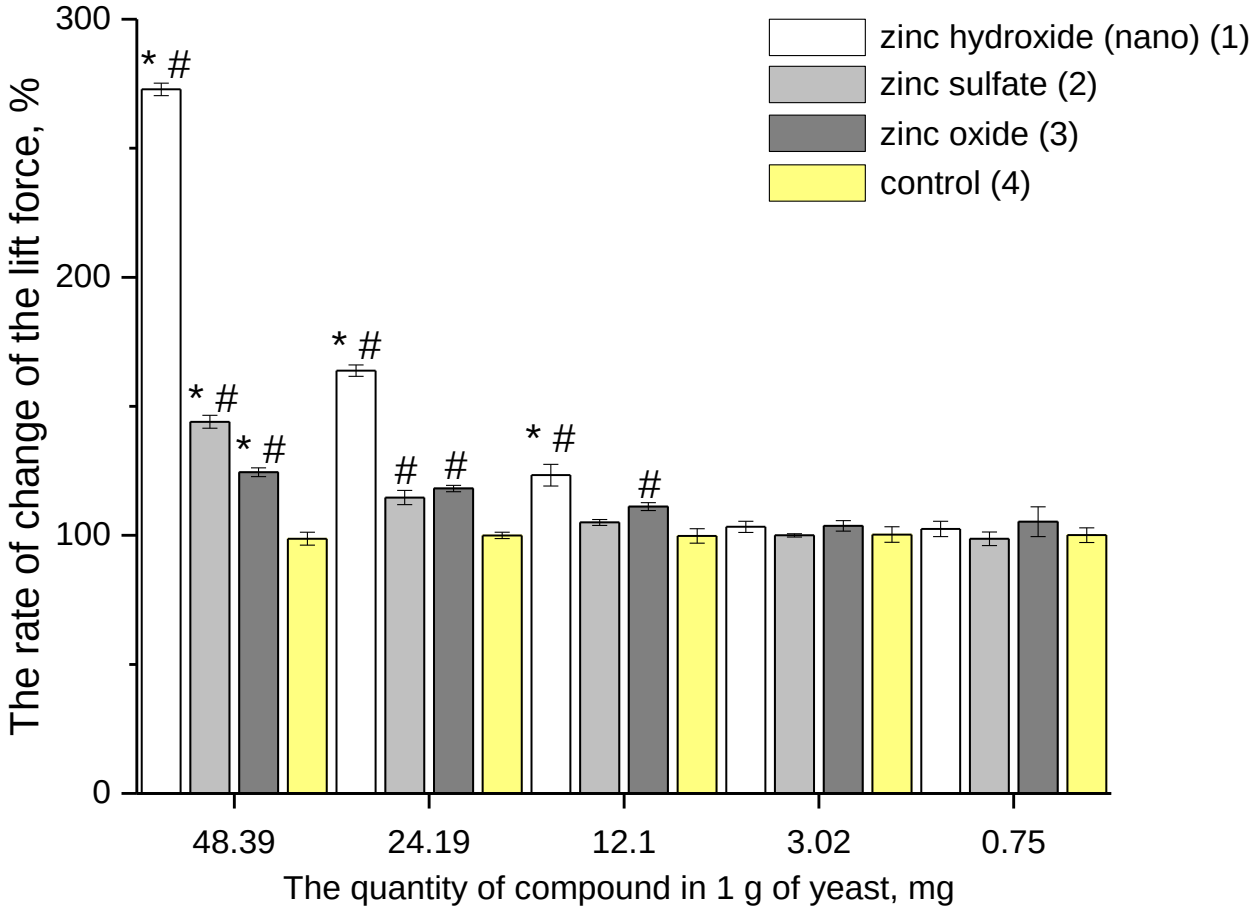

Figure 2. The rate of change of the lift force according to different mass concentrations of zinc compounds: 1 – nanoform of zinc hydroxide, 2 – soluble zinc sulfate, 3 – a commercial sample of zinc oxide, 4 – control group ($M \pm m$, p=0.05, * - differences have statistical significance in relation to subsequent group, # - differences have statistical significance against the control group).

Highest activity of zinc hydroxide in the experiments *in vitro* allows hypothesizing the activity of nanoforms *in vivo*. To assess the pharmacokinetic parameters of nanoscale zinc hydroxide of the experimental animals were administered enteral three dosage levels: 10, 50 and 100 mg/kg. For comparative evaluation in similar conditions, with an equal dose level, compound was administered for comparison of zinc sulfate. The results are shown in Figure 3.

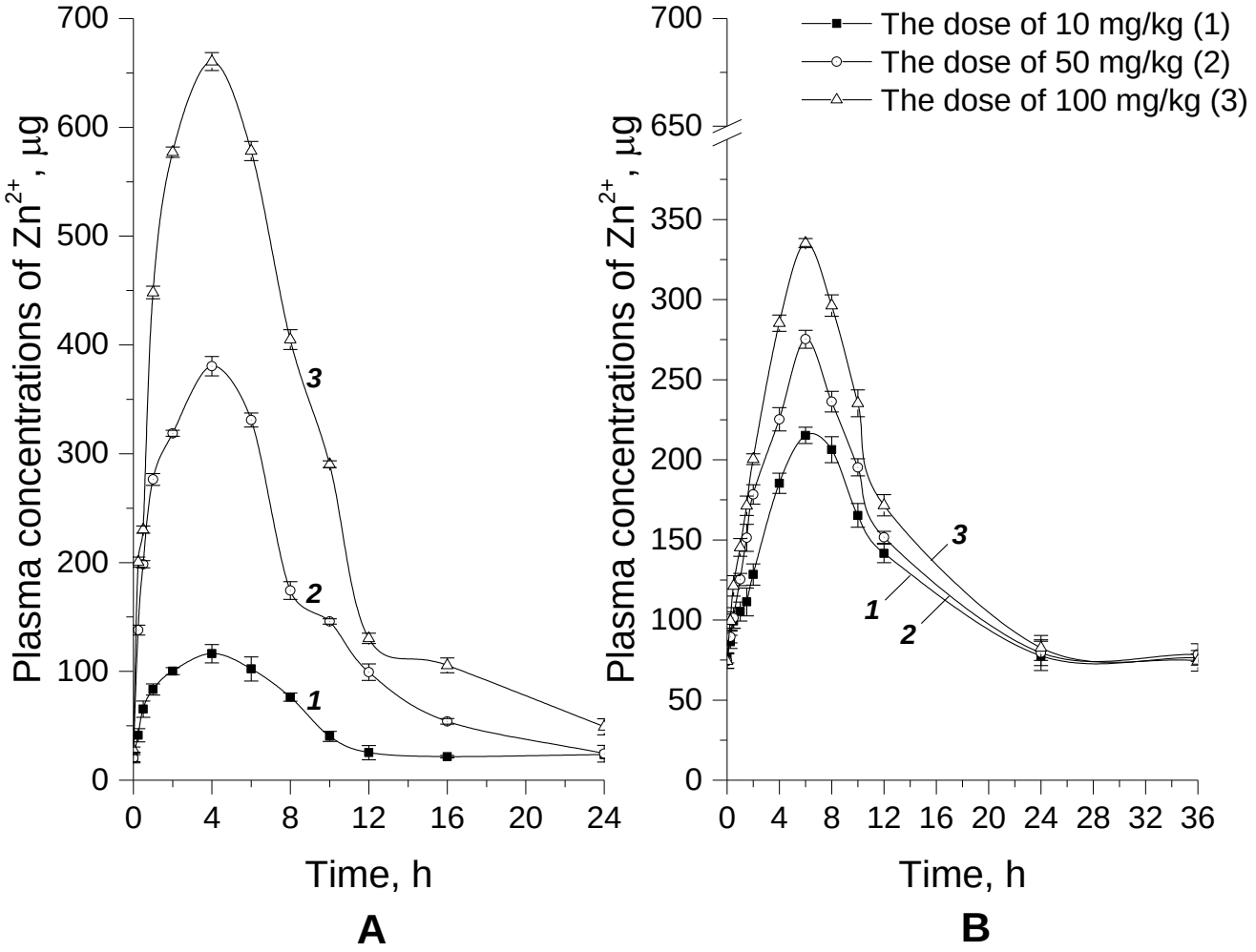

Figure 3. Pharmacokinetic curves nanoforms hydroxide (A) and zinc sulfate (B) when enteral administered in a dosage of: 10 (1), 50 (2) and 100 (3) mg/kg.

The main pharmacokinetic parameters were calculated by model independent method based on the pharmacokinetic curves: AUC_{0-t} (h×µg/ml), AUMC (h^2× µg/ml), Cl_T (ml/h), MRT (hour), C_{max} (µg/ml), t_{max} (h), $T_{1/2}$ (h), and C_{max}/AUC_{0-t} (1/hour), f_a (%).The results are shown in table 1.

Table 1.

Pharmacokinetic parameters of nanoforms of zinc hydroxide and soluble zinc sulfate after a single enteral introduction in three dosages ($M \pm m$; n=3 (per group)).

Parameters	Single enteral administration of nanoform of zinc hydroxide.			Single enteral administration of the dissolved zinc sulphate.		
	Dosage (mg/kg)			Dosage (mg/kg)		
	10	50	100	10	50	100
AUC_{0-t}, h×µg/ml	1040.47 ± 40.85	3580.90 ± 60.86	6430.28 ± 49.51	1260.14 ± 61.94	3540.66 ± 56.03	6070.54 ± 70.18
AUMC, h^2×µg/ml	1243.78 ± 39.36	3793.56 ± 77.23	6643.93 ± 52.97	1472.95 ± 68.93	3753.31 ± 55.99	6276.82 ± 73.17
Cl_T, ml/h	9.60 ±0.38	13.96 ± 0.24	15.55 ± 0.12	7.92 ±0.39	14.12 ± 0.22	16.47 ± 0.19
MRT, h	1.19 ± 0.01	1.06 ± 0.01	1.03 ± 0.01	1.17 ± 0.01	1.06 ± 0.01	1.03 ± 0.01
C_{max}, h	120.73 ± 6.47	390.58 ± 10.38	660.47 ± 10.17	169.72 ± 7.63	383.82 ± 25.78	553.86 ± 24.44
T_{max}, h	4	4	4	6	6	6
$T_{1/2}$, h	4.97 ± 0.15	5.92 ± 0.16	5.09 ± 0.12	5.60 ± 0.19	5.49 ± 0.40	5.58 ± 0.34
C_{max}/AUC, 1/h	0.12 ±0.01	0.11 ±0.01	0.10 ±0.02	0.13 ±0.01	0.11 ±0.02	0.09 ±0.02
F_a, %	30.33 ± 3.16	44.39 ± 4.52	42.47 ± 3.66	33.03 ± 3.55	42.67 ± 5.63	39.51 ± 2.12

The maximum concentration of Zn^{2+} in blood plasma after administration of compounds has the following trends: at a dose of 10 mg/kg C_{max} ($p<0.05$) higher for zinc sulfate than for nanoparticles of zinc hydroxide (169.72±7.63 μg/ml and 120.73±of 6.47 μg/ml) at a dose of 50 mg/kg groups did not have statistically significant differences (390.58±10.38 and 383.82±25.78 μg/ml) at a dose of 100 mg/kg nanoforms of zinc hydroxide ($p<0.05$) have higher maximum concentration in comparison with soluble zinc sulfate (660.47±10.17 μg/ml and 553.86±24.44 μg/ml). The time to maximum concentration for nanoforms of zinc hydroxide is 4 hours (regardless of the administered dose). This figure is higher than in groups which were introduced zinc sulfate – t_{max} in which the figure amounted to 6 hours. To demonstrate the quantitative assessment eliminable substances for each group was calculated total clearance (Cl_T). This parameter is comparable to nano-sized zinc hydroxide and compounds-comparison, which was introduced enteral method, increasing with increasing appropriate dosage: 9.60±0.38, 13.96±0.24, 15.55±0.12 and 7.92±0.39, 14.12±0.22, 16.47±0.19 ml/h. Values for average holding time (MRT) when intragastric introduction does not have statistical significant differences for zinc sulfate and zinc hydroxide and is not changed with increasing dosage. On the basis of area under the curve was determined the absolute bioavailability (f_a) in comparison with intravascular administration. Values for nanosized hydroxide zinc values for the three doses respectively were: 30.33±3.16, 44.39±4.52 and 42.47±3.66%. These parameters were calculated for the comparison compound of the sulphate of zinc: 30.03±3.55, 42.67±5.63 and 39.51±2.12%. Significantly ($p<0.05$) higher in the nanoforms of zinc hydroxide this parameter is defined for the dose of 100 mg/kg.

When entering the digestive tract, the nanoparticles are partially soluble in gastric juice to form zinc ions [18, 10]. The efficiency of dissolution depends on the particle size, determining the surface area that comes in contact with acid and from the spatial structure. Insoluble nanoparticles can pass through the intestinal wall without significant chemical transformations [19]. Introduction of small doses (10 mg/kg and 50 mg/kg) leads to a complete dissolution of the administered compound. This is evidenced by the maximum concentration that can be compared to zinc sulfate. With the introduction of a dose of 100 mg/kg slow dissolution of nanoforms in the stomach (spatial structure of particles of zinc hydroxide is described as a colloidal micelle, surrounded by a stabilizing shell, consisting of counterions). For this reason it is not dissolved in the nanoparticles are absorbed intact into the systemic circulation. This hypothesis was confirmed in experiments on rats. In carrying out that after the introduction of its nanoform deposits were found in the intestine [20]. The nature of absorption explains the more rapid time to maximum concentration of nanoforms in the blood plasma. Penetration into the systemic circulation in the form of particles excludes the time spent on the binding of zinc ions with proteins. Meanwhile, a part of zinc, converted in a dissolved form, provides a slower decay after reaching the maximum concentration.

Investigate pharmacokinetic properties of nanoforms of zinc hydroxide and soluble zinc sulfate under conditions of intravascular injection at doses similar to enteral infusion was performed for comparative performance. The pharmacokinetic curves are shown in Figure 4.

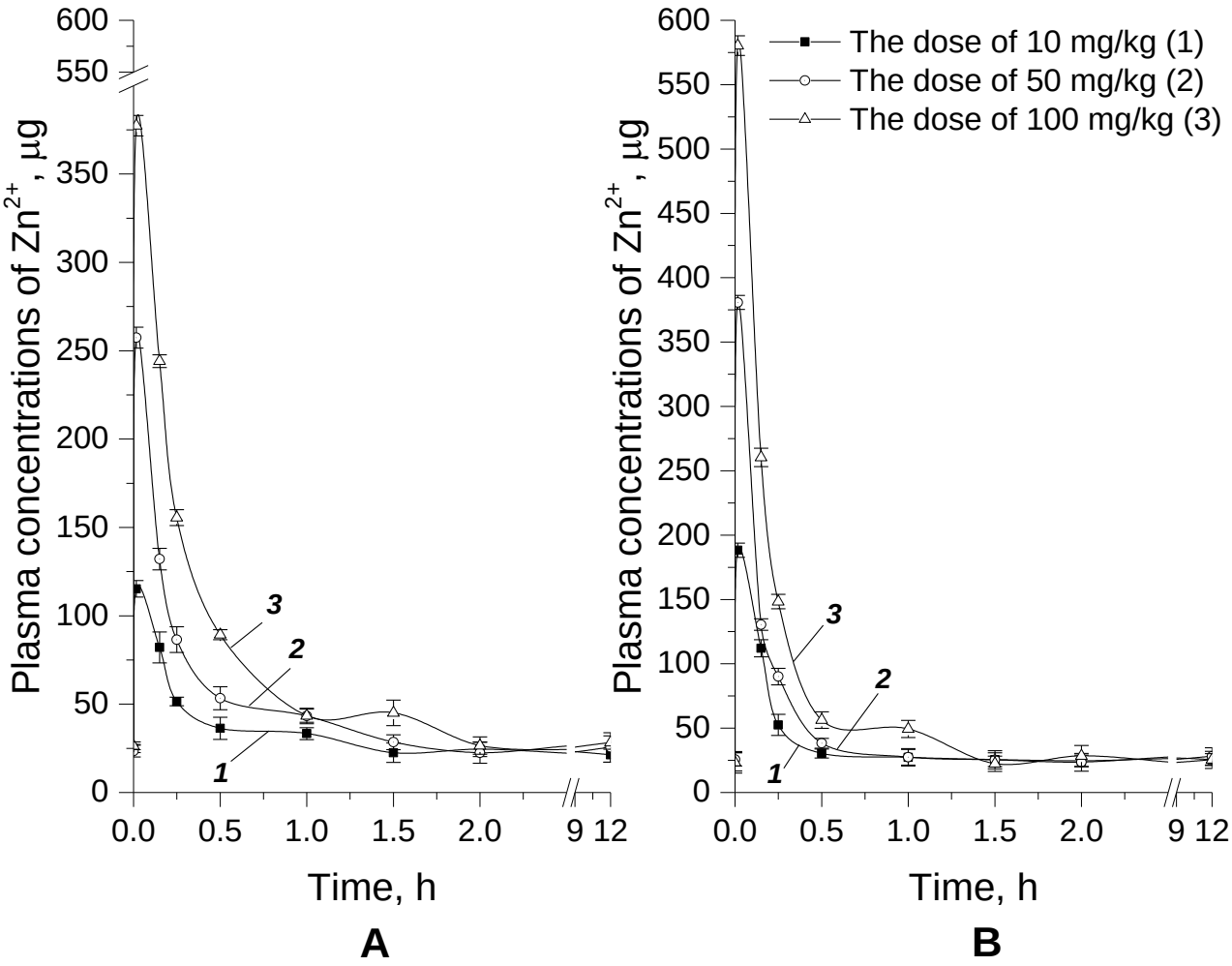

Figure 4. Pharmacokinetic curves nanoforms hydroxide (A) and zinc sulfate (B) when administered intravenously in a dosage: 10 (1), 50 (2) and 100 (3) mg/kg.

Based on the pharmacokinetic curves were calculated pharmacokinetic characteristics that given in Table 2.

Table 2

Pharmacokinetic parameters of zinc hydroxide nanoform and soluble zinc sulfate after single intravascular injection of three doses ($M \pm m$; n=3 (per group)).

Parameters	Single injection of nanoforms of zinc hydroxide. dosage (mg/kg)			Single injection of sulfate zinc. dosage (mg/kg)		
	10	50	100	10	50	100
AUC_{0-t}, h× µg/ml	34.13 ± 2.67	80.02 ± 9.45	150.72 ± 11.86	38.00 ± 2.48	82.13 ± 9.79	153.29 ± 10.13
AUMC, h^2× µg/ml	59.17 ± 1.52	114.26 ± 5.89	150.72 ± 11.86	64.59 ± 4.74	131.01 ± 10.31	257.01 ± 14.79
Cl_T, ml/h	291.86 ± 22.27	619.12 ± 73.81	660.77 ± 51.86	262.39 ± 17.41	603.29 ± 70.06	650.48 ± 42.17
MRT, h	1.00 ±0.05	1.01 ± 0.01	1.01 ± 0.01	1.03 ±0.01	1.00 ±0.02	0.99 ±0.01
C_{max}, h	114.73 ± 4.81	261.27 ± 4.43	396.11 ± 17.44	175.65 ± 10.68	382.13 ± 5.72	593.28 ± 11.41
T_{max}, h	0.016	0.016	0.016	0.016	0.016	0.016
$T_{1/2}$, h	0.34 ±0.10	0.44 ±0.12	0.39 ±0.04	0.13 ±0.01	0.14 ±0.03	0.14 ±0.01
C_{max}/AUC, 1/h	3.34 ± 0.36	3.23 ± 0.43	2.89 ± 0.42	4.42 ± 0.25	4.61 ± 0.60	4.26 ± 0.59

For all groups the maximum concentration of zinc ions Zn^{2+} was detected in the blood after 0.016 hours after drug administration. Further, there was a sharp decrease in the content of zinc ions in the plasma, with no statistically significant differences in comparison with the control group after 2 hours. These data are

consistent with the work of other researchers in which the time of occurrence of maximum concentration with intravenous administration of nanoparticles of zinc oxide was determined for 1 minute [12]. The maximum concentration of zinc ions in plasma ($p<0.05$) higher when enteral introduction of nanoforms (compared to intravenous) in dosages of 50 and 100 mg/kg (amount: 390.58±10.38 and 261.27±4.43 μg/ml, 660.47±10.17 and 396.11±17.44 μg/ml). When comparing the zinc levels in the plasma for enteral and intravenous administration of soluble zinc sulfate found no statistically significant differences (169.72 ± 7.63 and 175.65 ± 10.68 μg/ml, 383.82 ± 25.78 and 382.13 ± 5.72 μg/ml, 553.86 ± 24.44 and 593.28 ± 11.41 μg/ml, respectively, for a dosage 10, 50 and 100 μg/ml).

Registered contrast to the maximum concentrations after intravenous and intragastric administration due to several factors. First, it is the ability of particles to aggregate in the bloodstream or to adsorption on the surface of red blood cells. [21, 22]. Was carried out the separation of the plasma in order to determine the concentration of zinc, therefore, part of nanoforms of zinc hydroxide could remain on the surface of red blood cells. We are also confirmed (in the experiments on rats) that when assessing the distribution of zinc between plasma and erythrocyte mass, there is a significant preponderance of concentrations in the direction of the erythrocyte mass [20]. Second, the rapid recycling units of zinc hydroxide in the bloodstream, indicative of relatively higher parameters total clearance for the nanoforms.

To determine the anticipated target organs and to assess the dynamics of accumulation of trace element concentrations were determined in blood fractions of rats: plasma and erythrocyte mass, in the anticipated target organs: the testes and the liver (in terms of repeated administration of one dose).

Dynamics of accumulation of zinc ions in the blood plasma has a similar trend for each of the three groups: statistical significant increase in the concentration ($p<0.05$), which varies from lower levels Zn^{2+} after 120 hours (the cancellation period) and after the resumption of reception increases (figure 5). This effect is the break with the introduction of rats studied compounds and shows a high rate of redistribution to other organs of zinc, in excess contained in the plasma. It is noted for the group treated with nanoparticles, the reduction of concentration was minimum (120 hours), while in the groups, which were injected microparticles of ZnO and comparison compound, the concentration Zn^{2+} (4.20±0.12 and 4.22±0.17 μg/ml) returned to the values of the control group (3.70±0.13 μg/ml). The maximum concentration in the blood plasma of the treated group nanoform, at the end of the experiment has no statistical significant difference with group, receiving the comparison compound (5.63±0.19 and 6.08±0.12 μg/ml).

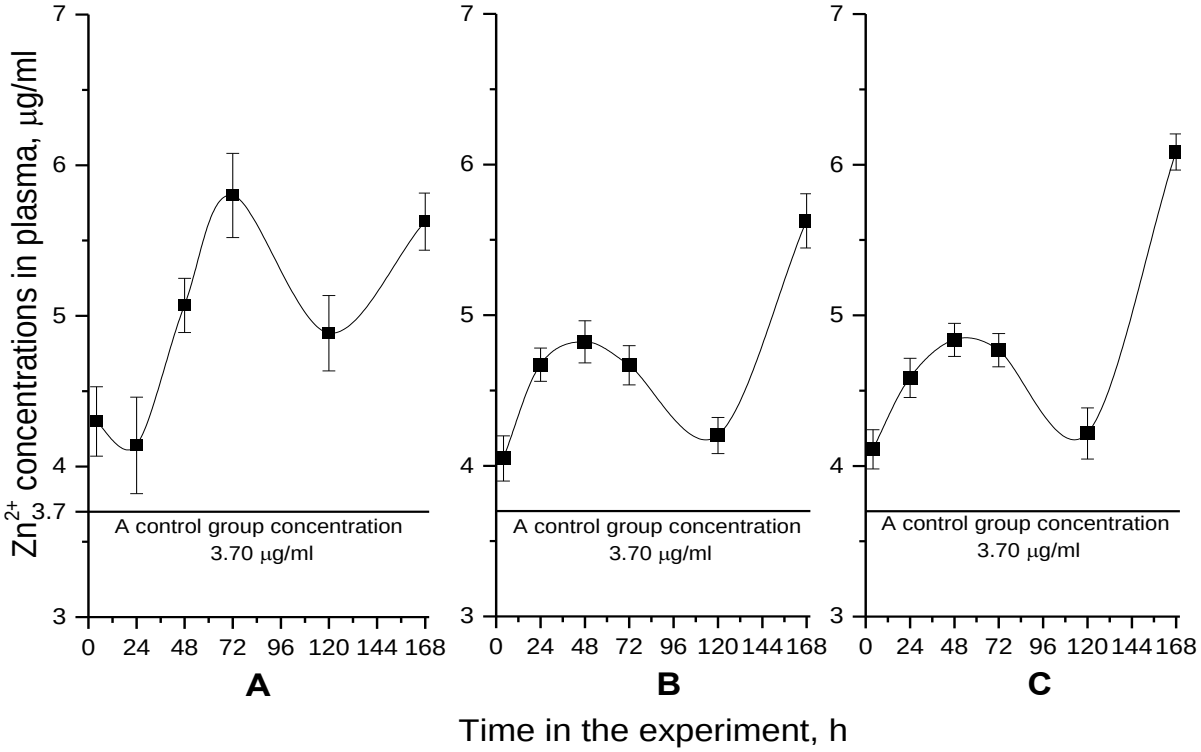

Figure 5. Dynamics of the accumulation: (μg/ml) in blood plasma of rats: group (a), treated with nanoform zinc hydroxide. Group (b) was treated with micro-sized zinc oxide. Group (c) treated with zinc sulfate.

Zinc concentration was determined in erythrocyte mass of blood (fig. 6). In group A (nanoform of zinc hydroxide) there is a significant increase of zinc concentration in erythrocyte mass after the second dose of compound (5.20±0,11 μg/g compared to 4.01±0,11 μg/g in the control group) and the lack of concentration decrease after 120 hours (during the break). The nature of changes in the level of zinc in red blood cell mass during the introduction of soluble $ZnSO_4$ has the statistical significance ($p<0.05$) and differed among each of the groups, decreasing in the series $Zn(OH)_2$ – ZnO – $ZnSO_4$. For zinc sulfate detected a relatively low ability to accumulate in red blood cell mass and is characterized by the reduction of zinc concentration to 4.24±0.17 μg/g at 120 hours (during the break). When resuming the introduction of the compounds value increases and by the end of the experiment is 5.12±0.11 μg/g. In the groups treated with nanoparticles $Zn(OH)_2$ and ZnO, these values are 10.13±0.11 μg/g and 8.58±0,21 μg/g.

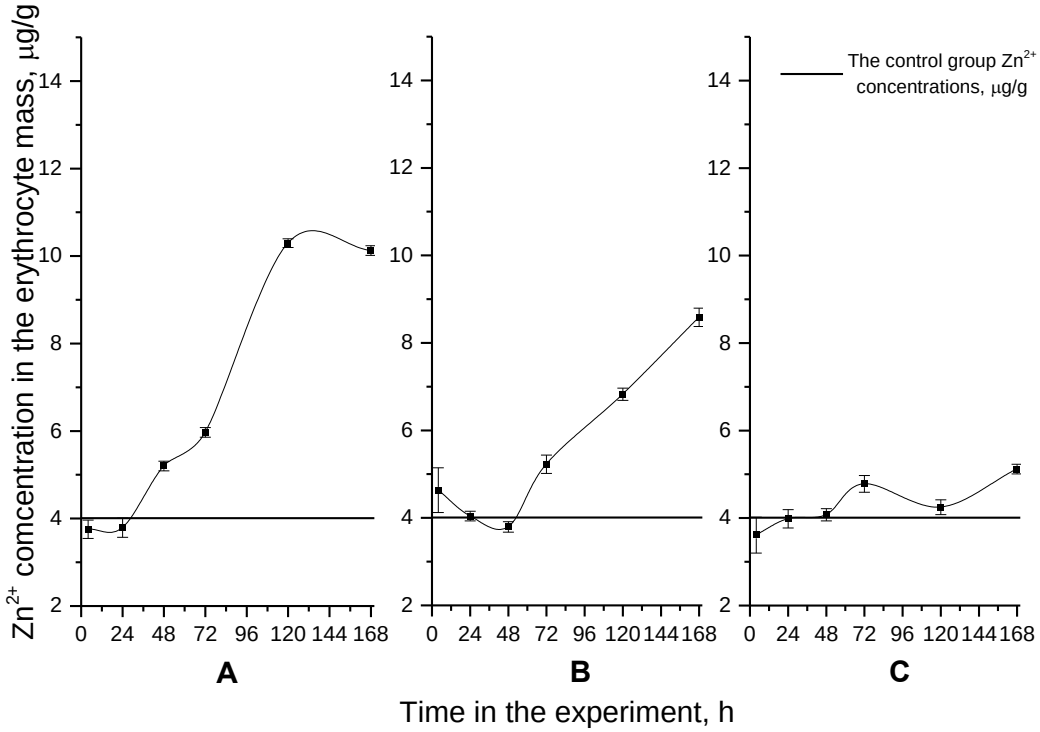

Figure 6. Dynamics of the accumulation Zn^{2+} (μg/g) in erythrocyte mass of blood of rats: Group (a) receiving nanoform zinc hydroxide. Group (b) treated with micro-sized zinc oxide. Group (c) treated with zinc sulfate.

Dynamics of bioaccumulation in erythrocyte mass (fig. 6), there is a significant increase in concentration after 24 hours (two cycles) after ingestion of nanoparticles of zinc oxide and decrease in zinc level within the cancellation period of the treatment. This result is due to two factors: first, the relatively low absorption of nanoparticles in the gut and second, the formation of a pool of zinc on the inner surface of the intestinal wall, as reported in the literature [23]. This was indirectly confirmed during the autopsy, which in the intestine of rats were found to have extensive deposits nanoforms. The total concentrations for groups A and b was higher than that of blood plasma, which determines the presence of adsorption of particles on the surface of red blood cells (this is consistent with literature data) [22].

The relatively low level of bioaccumulation of nanosized zinc hydroxide in liver tissues compared to the zinc sulfate is an advantage of the compounds (figure 7).

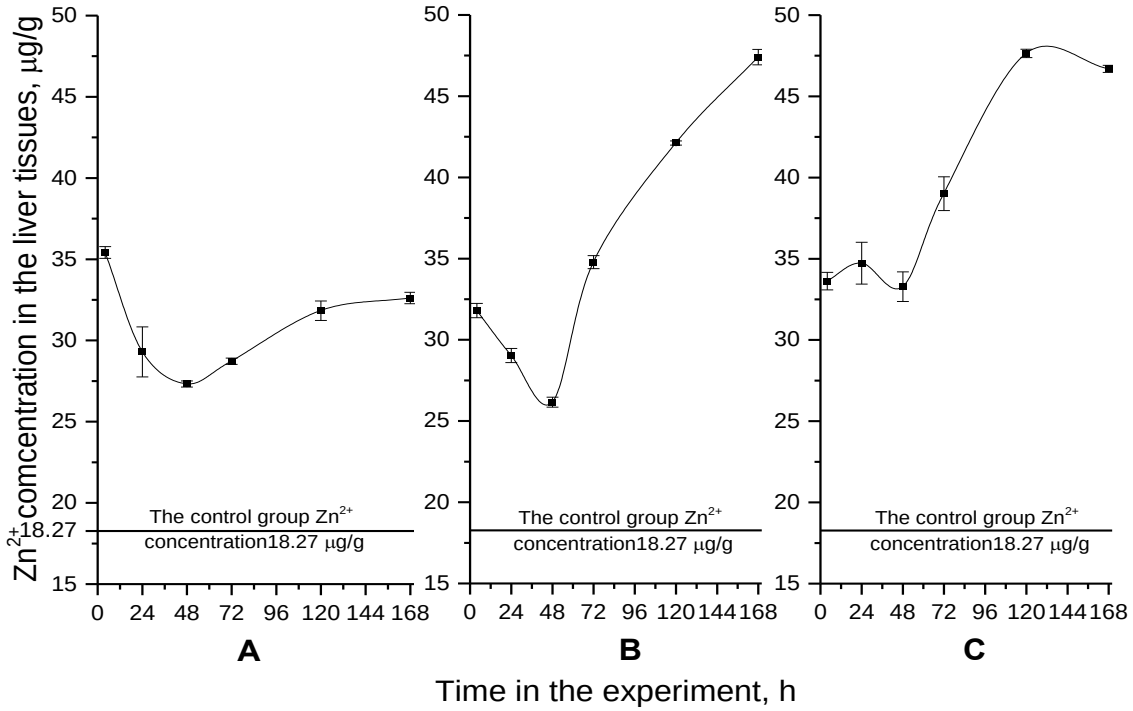

Figure 7. Dynamics of the accumulation Zn^{2+} (µg/g) in liver tissues of rats: Group (a) receiving nanoform zinc hydroxide. Group (b) treated micro zinc oxide. Group (C) treated with zinc sulfate.

The literature describes cases of hepatotoxicity because of active accumulation of zinc in liver tissue with the introduction of experimental animals of nanoparticles of zinc oxide [24]. In the interval of 4 hours has been a slight increase in zinc content. The increase subsequently decreases, and further noted a small increase. In the group where rats were administered zinc oxide, after 168 hours the concentration Zn^{2+} in the liver exceeds the concentration in the control group is 2.5 times. This result is consistent with the literature data [25]. For compounds-comparison ($ZnSO_4$) to the end of the experiment the obtained data exceeds 2.5 times the concentration of zinc in the liver compared with the control group.

The importance of the influence of zinc ions on the reproductive function for the chosen compounds was also assessed the results of accumulation in the tissues of the testes (figure 8).

There was a dramatic increase of zinc concentration in the testes in the group that received nanoparticles of zinc hydroxide. Through 168 hours indicator reaches a value of 10.24±0.21 µg/g. These values are comparable with the group that was administered the sulfate of zinc. Indicator by the end of the experiment was 10.34±0.14 µg/g. The increase of zinc concentration in the group C marked after 72 hours, and in group A the increase was observed immediately after the introduction. The group that received microparticles of zinc oxide, shows a slight increase in the concentration of zinc ions in the testes, which remains at the same level throughout the experiment, and after 168 hours is 4.06±0.12 µg/g in the control group, the rate of growth of the concentration of zinc ions in the testes is 3.67±0.12 µg/g. Showed a high tendency to the accumulation of nanoscale compounds of zinc, comparable to the soluble ionized compound and is related to the size of the particles that overcome hematoma testicular barrier consisting of Sertoli cells [26]. For zinc sulfate, which is absorbed in form of ions, requires binding with proteins-carriers and the completion of a number of other lengthy stages, with the result that there may be an accumulation of only 120 hours. Polling transmission hematoma testicular barrier does not give ions of the zinc to penetrate the seminiferous tubules while increasing the overall level of zinc in the body. [27]. Microparticles of ZnO cannot effectively overcome the barrier, consisting of interstitial cells.

Conclusion.

The first stage was reproduced by the Sol-gel method of synthesis. The results were obtained for the gel containing nanoparticles of zinc hydroxide. The study of size distribution it was found that 80% of the particles belong to the range 2-3 nanometer.

Received nanoform was studied *in vitro* in comparison with soluble zinc sulphate and zinc oxide (commercial sample on the test culture *S.cerevisiae.* The data obtained demonstrate a high level of inhibition

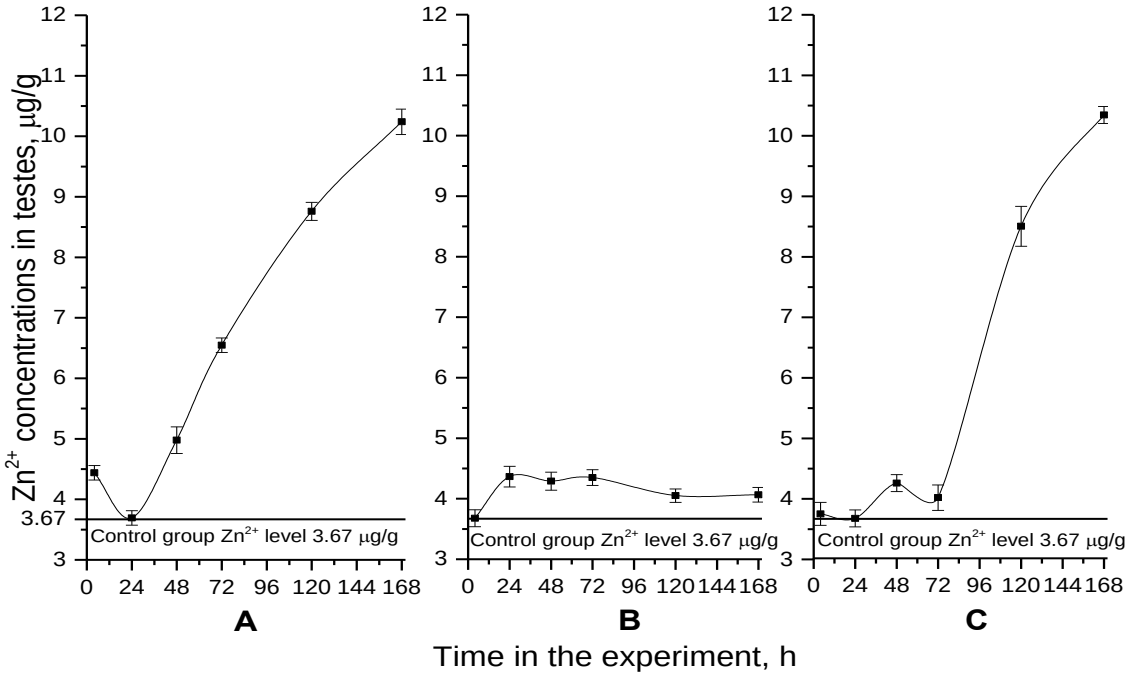

Figure 8. Dynamics of the accumulation Zn^{2+} (microgram/gram) in tissues of the testes of rats: Group (A) receiving nanoform zinc hydroxide. Group (b) treated with micro-sized zinc oxide. Group (c) treated with zinc sulfate.

of the complex of the alcoholic fermentation, enzymes in yeast cells by nanforms. This made the assumption of a high biological activity of nanoparticles of zinc hydroxide due to more intensive transport across the plasma membrane of cells.

In the course of the pilot study in rabbits was determined the main parameters of the pharmacokinetics of zinc in the introduction to nanoforms of zinc hydroxide and form soluble zinc sulfate (comparison compound). The time to reach maximum concentration of Zn^{2+} in enteral administration of nanoform was 4 hours, which is faster than that of zinc sulfate - 6 hours. The total clearance of nanoparticles increases with dose increases, amounting to 9.60±0.38, 13.96±0.24, 15.55±0.12 ml/h, half-life have not a statistically significant change. The maximum concentration of zinc ions in the blood plasma when intravenously nanoforms hydroxide ($p<0.05$) lower compared to the enteric introduction and is 261.27±4.43 and 390.58±10.38 μg/ml at a dose of 50 mg/kg, 396.11±17.44 and 660.47±10.17 μg/ml for doses of 100 μg/g. It also identified the parameters absolute bioavailability for nanoforms of zinc hydroxide: 30.33±3.16, 44.39±4.52 and 42.47±3.66%, respectively, for doses of 10, 50 and 100 mg/kg. We can state that in the dosages of 10, 50 mg/kg pharmacokinetic properties of compared compounds do not have significant differences, which are shown only at 100 mg/kg.

A comparative study of accumulation parameters of nanoparticles in blood fractions, liver and testes identified target organs with affinity to the nanoscale hydroxide zinc compared to zinc sulfate. Erythrocyte mass of the testes is an advantage of the obtained compounds. A low level of accumulation in the liver compared with the comparison compound may indicate smaller forms of hepatotoxicity, which is typical for zinc sulfate.

References.

1. Kumssa D.B., Joy E.J.M., Ander L., Watts M.J., Young S.D., Walker S., Broadley M.R. Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. Scientific reports. №5. (2015): 1-11. [PubMed] [Full text]

2. Lim S.S., Vos T., Flaxman A.D., Danaei G., Shibuya K., Adair-Rohani H., Amann M., Anderson H.R., Andrews K.G. [*et al*]. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study. The Lancet. Vol. 380. (2012): 2224-2260. [PubMed] [Full Text]

3. Brown K.H., Peerson J.M., Rivera J., Allen L.H. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition. Vol. 75. (2002): 1062-1071. [PubMed] [Full Text]

4. Maret W. Zinc biochemistry: from a single zinc enzyme to a key element of life. Advances in Nutrition. Vol. 4. (2013): 82-91. [PubMed] [Full Text]

5. Khabarov A.A., Budko E.V., Lushov K.A., Gorbacheva L.A., Eltsova N.O. Zinc: topicality and characteristics of dietary supplements (review). Modern problems of science and education. Vol. 3. (2012): 361-369. [eLibrary] [Full Text]

6. Brown K.H., Hambidge K.M., Ranum P. Zinc fortification of cereal flours: current recommendations and research needs. Food and Nutrition Bulletin. Vol. 31. (2010): 62-74. [PubMed]

7. Whittaker P. Iron and zinc interactions in humans. The American Journal of Clinical Nutrition. Vol. 68. (1998): 442S-446S. [PubMed]

8. Khaliullina S.V. Clinical significance of zinc deficiency in the child (literature review). Bulletin of Contemporary Clinical Medicine. Vol. 3. (2013): 72-78. [eLibrary] [Full Text]

9. Khadka P., Ro J., Kim H., Kim I., Kim J.T., Kim H., Cho J.M., Yun G., Lee J. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian Journal of Pharmaceutical Sciences. Vol. 9. (2014): 304-316. [Full Text]

10. Baek M., Chung H.E., Yu J., Lee J.A., Kim T.H., Oh J.M., Lee W.S., Paek S.M., Jeong J., Choy J.H., Choi S.J. Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles. International Journal of Nanomedicine. Vol. 7. (2012): 3081-3097. [PubMed] [Full Text]

11. Choi S.J., Choy J.H. Biokinetics of zinc oxide nanoparticles: toxicokinetics, biological fates, and protein interaction. International Journal of Nanomedicine. Vol. 9. (2014): 261-269. [PubMed] [Full Text]

12. Kim M.K., Lee J.A., Jo M.R., Choi S.J. Bioavailability of Silica, Titanium Dioxide, and Zinc Oxide Nanoparticles in Rats. Journal of Nanoscience and Nanotechnology. Vol. 6. (2016): 6580-6586. [PubMed]

13. Raspopov R.V., Buzulukov Yu.P., Marchenkov N.S., Solovyev V.Yu., Demin V.F., Kalistratova V.S., Gmoshinsky I.V., Khotimchenko S.A. The bioavailability zinc oxide nanoparticles. Study methods of radionuclide indicator. Nutrition issues. Vol. 6. (2010): 14-18. [eLibrary]

14. Stehlik-Tomas V., Zetic V.G., Stanzer D., Grba S., Vahcic N. Zinc, copper and manganese enrichment in yeast Saccharomyces cerevisae. Food Technology and Biotechnology. Vol. 42. (2004): 115-120. [ResearchGate] [Full Text]

15. Lui X.F., Supek F., Nelson N., Culotta V.C. Negative control of heavy metal uptake by the Saccharomyces cerevisiae BSD2 gene. The Journal of Biological Chemistry. Vol. 272. (1997): 11763-11769. [PubMed]

16. Ivask A., Juganson K., Bondarenko O., Mortimer M., Aruoja V., Kasemets K., Blinova I., Heinlaan M., Slaveykova V., Kahru A. Mechanisms of toxic action of Ag, ZnO and CuO nanoparticles to selected ecotoxicological test organisms and mammalian cells in vitro: a comparative review. Nanotoxicology. Vol. 8. (2014): 57-71. [PubMed] [Full Text]

17. Xia T., Kovochich M., Brant J., Hotze M., Sempf J., Oberley T., Sioutas C., Yeh J.I., Wiesner M.R., Nel A.E. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Letters. Vol. 8. (2006): 1794-1807. [PubMed] [Full Text]

18. Song W., Zhang J., Guo J., Zhang J., Ding F., Li L., Sun Z. Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. Toxicology Letters. Vol. 199. (2010): 389-397. [PubMed]

19. Kotenko K.V., Belyaev I.K., Buzulukov Yu.P., Bushmanov A.Yu., Demin V.F., Zhorova E.S., Kalistratova V.S., Marchenkov N.S., Nisimov P.G., Raspopov R.V., Soloviev V.Yu. Experimental Study of Zinc Oxide-labelled Nanoparticles Biokinetics in Rats after Single Oral Administration. Medical radiology and radiation safety. Vol. 2. (2011): 5-10. [eLibrary]

20. Larin S.L., Budko E.V., Khabarov A.A., Lipatov V.A., Zvyagintseva A.R. Experimental study of zinc accumulation dynamics in intragastric administration of $Zn(OH)_2$ nanoparticles and ZnO microparticles. Kursk scientific and practical bulletin «Man and its health». Vol. 3. (2016): 100-106. [eLibrary] [Full Text]

21. Saptarshi S.R., Duschl A., Lopata A.L. Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle. Journal of Nanobiotechnology. Vol. 11. (2013): 11-23. [PubMed] [Full Text]

22. Šimundić M., Drašler B., Šuštar V., Zupanc J., Štukelj R., Makovec D., Erdogmus D., Hägerstrand H., Drobne D., Kralj-Iglič V. Effect of engineered TiO2 and ZnO nanoparticles on erythrocytes, platelet-rich plasma and giant unilamelar phospholipid vesicles. BMC Veterinary Research. Vol. 9. (2013): 7-20. [PubMed] [Full Text]

23. Ko J.W., Hong E.T., Lee I.C., Park S.H., Park J.I., Seong N.W., Hong J.S., Yun H.I., Kim J.C. Evaluation of 2-week repeated oral dose toxicity of 100 nm zinc oxide nanoparticles in rats. Laboratory Animal Research. Vol. 31. (2015): 139-145. [PubMed] [Full Text]

24. Mansouri E., Khorsandi L., Orazizadeh M., Jozi Z. Dose-dependent hepatotoxicity effects of Zinc oxide nanoparticles. Nanomedicine Journal. Vol. 2. (2015): 273-282. [Full Text]

25. Watson C.Y., Molina R.M., Louzada A., Murdaugh K.M., Donaghey T.C., Brain J.D. Effects of zinc oxide nanoparticles on Kupffer cell phagosomal motility, bacterial clearance, and liver function. International Journal of Nanomedicine. Vol. 10. (2015): 4173-4184. [PubMed] [Full Text]

26. Talebi A.R., Khorsandi L., Moridian M. The effect of zinc oxide nanoparticles on mouse spermatogenesis. Journal of Assisted Reproduction and Genetics. Vol. 30. (2013): 1203-1209. [PubMed] [Full Text]

27. Franca L.R., Ghosh S., Ye S.J., Russell L.D. Surface and surface-to-volume relationships of the Sertoli cell during the cycle of the seminiferous epithelium in the rat. Biology of Reproduction. Vol. 49. (1993): 1215-1228. [PubMed] [Full Text]