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EXPERIMENTAL PHARMACOLOGY

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Avdeeva N.V.1, Kulikov A.L.2, Pokrovskii M.V.3, Avtina T.V.4

PHARMACOKINETIC STUDIES OF NEW ANTIPARKINSONIAN DRUG RAPITALAM

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Abstract
Parkinson's disease is the most common neurodegenerative disorder after Alzheimer's disease. The aim of this study was to investigate the pharmacokinetic parameters of the mGluR4 receptor blocker Rapitalam on rabbits. There was developed the method of the quantitative determination of Rapitalam in the blood plasma of rabbits using high performance liquid chromatography with tandem mass spectrometric detection. The study was performed on 12 rabbits (males, weighing between 3,300 to 3,500 g). In intragastric dosing of the substance was administered using a gastric tube in the form of suspension in water 0.9 mg/ml, 9 mg/ml, and 90 mg/ml at a dose of 0.3 mg/kg, 3 mg/kg and 30 mg/kg. After the administration of the substance blood was sampled through a catheter in a volume of 0.5 ml in polypropylene tubes containing 20 µl of 5% EDTA before and 10, 15, 30, 60, 120, 240, 480, 1440 minutes after administration. The mean absorption time (MAT) of Rapitalam was 268.1 minutes or 4.5 hours. The half-life is longtime and it was 176.4 minutes (2.9 hours) for intravenous and 362.2 minutes (6.0 hours) for intragastric administration. The absolute bioavailability of the intragastric dosing was 26.8%. The main pharmacokinetic parameters of the substance was established on rabbits that allow you to optimize the future use of it's as a potential drug for the treatment of Parkinson's disease.

Key words: Rapitalam, Parkinson's disease, the blood plasma of rabbits, high performance liquid chromatography, pharmacokinetic, metabotropic glutamate receptors.

Introduction
Parkinson's disease belongs to a group of neurodegenerative diseases of the brain. The main symptoms of Parkinson's are the loss of coordination, constraint and slowness when walking, tremor (shaking) of hands, feet, chin. Most often Parkinson's disease affects the elderly [1]. The pathogenesis of the disease is the insufficient synthesis of dopamine in the substantia nigra and striatum. Dopamine replacement therapy is currently the primary approach in the treatment of Parkinson's disease. However, levodopa does not slow down the continued degeneration of dopaminergic neurons, the functional activity of which causes the conversion of levodopa into dopamine by action of DOPA decarboxylase [5]. Also drugs of levodopa group have a large number of side effects, and over time, the dosage must be increased, otherwise they lose
effectiveness. Therefore, the attending physician of a patient suffering from Parkinson's disease has two main tasks: to suspend the death of ganglion containing dopamine, and reduce the symptom load of disease [1]. Progress in the understanding of the anatomy and function of basal ganglia has provided an opportunity for the development of new drugs for the treatment and slowing the progression of Parkinson's disease [3]. Although they are trying to cure Parkinson's disease, the experts can only partially eliminate the symptoms but not the cause itself. Today, all the efforts of scientists aimed at finding drugs that will not only soften the symptoms of the disease, but also stopped the degenerative processes responsible for its progression. So glutamate receptors have been proposed as promising therapeutic targets, since the effects on them allow you to change both normal and pathological neurotransmission characteristic of the Parkinsonian brain.

The above data indicate the feasibility of preclinical study of Rapitalam, mGluR4 receptor modulator, to create on its basis drugs that have anti-Parkinsonian effect [6].

The aim of this study was to investigate the pharmacokinetic parameters of the mGluR4 receptor blocker Rapitalam on rabbits.

Materials and methods

There was studied a Rapitalam substance, white powder. Rabbits are common animals used in studies of pharmacokinetics in accordance with the "Manual on experimental (preclinical) study of new pharmacological substances" [6]. Moreover, these animals are economically beneficial, because in the preliminary catheterization of the animals, to take samples of blood from each animal at all time points. The study was performed on 12 rabbits (males, weighing between 3,300 to 3,500 g). During the period of adaptation of animals, which is lasted 7 days, there was carried out daily inspection of their external condition. For the study there were selected rabbits with no signs of abnormalities in appearance. All animals were kept in separate rooms. Rabbits were kept by two in steel lattice coops. After a period of adaptation the animals were catheterized in the right ear vein and kept individually. The basic rules of keep and care consistent with the standards given in the manual • Guide for the care and use of laboratory animals. The National Academy press. –Washington, D.C. 2011 [9] and regulations approved by all-Union State Standard 31886-2012 "Principles of good laboratory practice". 12 hours before the start of the experiment, catheterized animals were deprived of feed with free access to water. The test substance was administered on the third day after catheterization. Intravenous dosing the test substance was administered bolus of 6 rabbits in the ear vein in the form of a solution 9 mg/ml in propylene glycol at a dose of 3 mg/kg. In intragastric dosing of the substance was administered using a gastric tube in the form of suspension in water 0.9 mg/ml, 9 mg/ml, and 90 mg/ml at a dose of 0.3 mg/kg, 3 mg/kg and 30 mg/kg. After the administration of the substance blood was sampled through a catheter in a volume of 0.5 ml in polypropylene tubes containing 20 µl of 5% EDTA before and 10, 15, 30, 60, 120, 240, 480, 1440 minutes after administration. Blood plasma was separated by centrifugation at 5600 g for 10 min and stored until analysis at -70 C.

Sample preparation blood plasma samples was performed by protein precipitation with methanol. To do this, into a test tube type "Eppendorf" with a capacity of 1.5 ml there were added 0.2 µl of plasma and 50 µl of internal standard and mixed, after there was added 500 µl of MeOH and shaken on a shaker for 15 minutes. Then there was performed the extraction of the analyte into ultrasonic bath for 15 minutes. Then the samples were centrifuged at 13000 rpm and 4 ºC temperature for 30 minutes. The supernatant is carefully decanted into vials for chromatography and analyzed.

In quality control solutions and to construct the calibration curve as placebo there was used zero blood plasma of rabbits. Zero samples of rabbits’ blood plasma were prepared in addition to calibration standards to confirm the selectivity of the method. The quality control solutions were analyzed during the researches and they found the error between an administered and found quantity of the analyte, compared with margins that served as a validation of a significance of the obtained results.

Rapitalam concentration in the blood plasma of rabbits was determined using the previously developed method of high performance liquid chromatography with tandem mass spectrometric detection which has high sensitivity and selectivity, that allows to determine low concentrations of drug (at the level of ng/ml) in various biological matrices [13]. Brief characteristic of the method is given in table 1.
The conditions of analysis.

<table>
<thead>
<tr>
<th>Apparatus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid chromatograph</td>
<td>Thermo Scientific Dionex UltiMate 3000 RS</td>
</tr>
<tr>
<td>Detector</td>
<td>Thermo Scientific Velos Pro c HESI.</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Chromatographic conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Guard column</td>
<td>Zorbax Eclipse XDB C18 12.5×3.0 mm with a particle size of 5.0 µm</td>
</tr>
<tr>
<td>Column</td>
<td>Acclaim™ 120 C18 150×2.1 mm with a particle size of 5.0 µm</td>
</tr>
<tr>
<td>Separate mode</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>5 mM Ammonium acetate +0.1% Formic acid: MeCN (60:40)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.3 mL/min</td>
</tr>
<tr>
<td>Temperature of samples</td>
<td>5 ºC</td>
</tr>
<tr>
<td>Temperature of column</td>
<td>40 ºC</td>
</tr>
<tr>
<td>Volume of injection</td>
<td>1 µL</td>
</tr>
<tr>
<td>Retention time of Rapitalam</td>
<td>about 7 min</td>
</tr>
<tr>
<td>Retention time of IS</td>
<td>about 4 min</td>
</tr>
<tr>
<td>Ionization type</td>
<td>ESI «+»</td>
</tr>
<tr>
<td>Mass transformation</td>
<td>Rapitalam: 383.84→367.0; IS: 244.7→130.94.</td>
</tr>
<tr>
<td>Temperature of source</td>
<td>300 ºC</td>
</tr>
<tr>
<td>Voltage across source</td>
<td>3000 V</td>
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</tbody>
</table>

The remaining parameters are in accordance with an automatic optimization tool.

Main pharmacokinetic parameters were calculated in accordance with the methodological recommendations for conducting preclinical studies of drugs under the editorship of A. N. Mironov [2]. Due to Microsoft Office Excel 2010 on the basis of the experimentally obtained data there were calculated the pharmacokinetic parameters. Outliers in each time point were identified using a statistical test of Grubbs [4]. This method showed good and accurate results [10, 11, 12]. Arithmetic mean values and the coefficient of variation (CV) were calculated in 6 animals.

The peak areas of the analyte and internal standard 2 were calculated by specialized software Xcalibur 2, then the data were transferred to a Microsoft Office Excel 2010, where we calculated the equation of the calibration curve, statistically evaluated deviation, graphically displayed the results [13]. Rapitalam concentration in the studied samples was calculated in Microsoft Office Excel 2010 from the calibration curve. Outliers in each time point were identified using a statistical test of Grubbs [4]. If any sample value of Z was greater than the critical value for a given number of dimensions N, this sample was excluded from further calculation of pharmacokinetic parameters. So, for N=6 the critical value of Z is equal to 1.89, so samples with Z> 1.89 were considered outliers.

**Research results**

Into BelSU Clinical and Preclinical Studies Centre there was studied the pharmacokinetic of Rapitalam in the blood plasma of rabbits after a single intravenous administration in dose of 3 mg/kg and intragastric administration in the dose of 30 mg/kg. After analyzing the obtained results, it was identified that after intragastric administration the maximum concentration of Rapitalam in the blood plasma of rabbits is achieved after an average of 480 minutes (4.0 hours) (Fig. 1). The half-life is longtime (362.2 minutes or 6.04 hours). The mean absorption time (MAT) of Rapitalam was 268.1 min (4.47 hours). The absolute bioavailability fₐ (%) of Rapitalam after intragastric administration to rabbits was 26.8 %.
The linearity of the pharmacokinetic of Rapitalam was studied after a single intragastric administration of the drug to rabbits in three increasing dosages of 0.3 mg/kg, 3.0 mg/kg and 30 mg/kg. Based on these data, there was the hypothesis of linearity of the pharmacokinetic of Rapitalam. To test this hypothesis there was assessed statistically significant departures from zero the intercept AUC\(_{(0-1440)}\). The calculation is presented in figure 2. The results showed that the free member is insignificant different from zero and the hypothesis should be considered correct.

To further evaluate the linearity there were constructed concentration influence curves, normalized for dose, from time. The results are presented in figure 3.
From figure 3 it can be seen that the concentrations, normalized by dose, for the different dosages have the same nature and similar value. It is observed excess of maximum concentration for the dose of 0.3 mg/kg over others. This deviation should be a consequence of the higher solubility of a low dose in the fluid of the gastrointestinal tract, at low concentration the effect of solubilization and uniform distribution of Rapitalam throughout the volume. At high concentrations, due to the hydrophobic properties of the test substance, there is observed the effect of agglomeration between the particles, which causes differences in normalized concentrations. It should be noted that the dependence of the AUC$_{(0-1440)}$ on dose is linear, and it gives grounds to confirm the linearity of the studied range of Rapitalam in the range from 0 to 30 mg/kg for rabbits.

Conclusions

1. Performed research of the main pharmacokinetic parameters of Rapitalam allowed to develop a method of quantitative determination of this substance in the blood plasma of rabbits.

2. The results obtained in the study of pharmacokinetic of Rapitalam showed that the decrease in its concentration in blood plasma of treated animals is quick in a biexponential manner. The mean absorption time (MAT) of Rapitalam was 268.1 minutes or 4.5 hours. The half-life is long time and it was 176.4 minutes (2.9 hours) for intravenous and 362.2 minutes (6.0 hours) for intragastric administration. The absolute bioavailability of the intragastric dosing was 26.8%. The maximum concentration of Rapitalam was observed on average after 4 hours.

3. The study of the linearity of the pharmacokinetic showed that the response/dose in a dosing interval from 0 to 30 µg/kg in rabbits has a linear dependence.

References


ASSESSMENT OF THE DNA DAMAGE LEVEL IN PERIPHERAL BLOOD LEUKOCYTES OF MICE TREATED ORALLY WITH RAPITALAM IN ACUTE AND THERAPEUTIC DOSES

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Abstract
Rapitalam is a drug that is a modulator of the mGlur4 receptor - a kind of metabotropic glutamate receptors. Into BelSU Clinical and Preclinical Studies Centre there was performed experimental research, which carried out the identification and quantitative assessment of alkali-labile sites and DNA strand breaks in leukocytes of peripheral blood of male mice treated with Rapitalam. The method is based on the assessment of the integrity of DNA in leukocytes of the whole blood of animals. Rapitalam was administered to animals orally according to 2 schemes: a single acute dose (413 mg/kg, which corresponds to 1/5 LD50) dose and once daily in a therapeutic dose (3 mg/kg) for 4 days. For analysis we used peripheral blood of mice. As an indicator of DNA damage there was used the value of %TDNA. The results of this study established that the level of DNA damage of blood leukocytes (%TDNA) in the groups with acute dose of Rapitalam statistically significantly different from those values in control group of animals, indicating the presence of DNA-damaging activity of Rapitalam in the acute dose. Analysis of the level of DNA damage of blood leukocytes (%TDNA) in groups of animals treated with therapeutic dose of Rapitalam showed significant differences between animals treated with solvent (dimethyl sulfoxide) and animals treated with Rapitalam dissolved in DMSO. Conspicuous is the fact that there is observed a significant reduction in DNA damage in the therapeutic dose of Rapitalam as compared to the acute dose and the group receiving only the solvent. This suggests that Rapitalam in a therapeutic dose can influence on the processes of intracellular metabolism and acts as a protector.

Key words: Rapitalam, Parkinson's disease, metabotropic glutamate receptors, mGluR4 receptor modulators, DNA strand breaks in leukocytes, alkali-labile sites

Introduction
Rapitalam is a mGluR4 receptor modulator. Mglur4 is a kind of metabotropic glutamate receptors. This group of receptors, as the name implies, doesn’t "open" for the current of ions through the membrane of the neuron after activation and exerts its effect indirectly by intracellular signal molecules – second messengers. Metabotropic receptors are divided into three groups depending on their mechanism of action, homology of structure and list of selective ligands (1 – mGluR1, mGluR5; 2 – mGluR2, mGluR3; 3 – mGluR4, mGluR6, mGluR7, mGluR8). Groups of receptors differ in their mechanism of action. Receptors of the first group are associated with Gq-protein. Other groups of glutamate receptors, second and third, make with Gi-protein. It means that the activation of these receptors blocks the function of adenilate cyclase, which in the active state converts ATP into cAMP. Consequently, the work of cAMP protein kinase stops and a phosphorylation pathway that modify the homeostasis of calcium doesn’t start [1, 7].

Based on the foregoing, we can understand the differences in the effects of these receptors: activation receptors of 1 group leads to increasing of the activity of NMDA and AMPA receptors is increased (i.e. by increasing synaptic density), but also susceptibility to excitotoxicity increases; activation receptors of 2 and 3 groups, on the contrary, leads to decrease the activity and density of ionotropic receptors and decreases the likelihood of excitotoxicity [8].

Thus glutamate receptors play a huge role in the regulation of functioning and development of the nervous system. For example, glutamate plays a role in neuronal death in hypoxia – in such circumstances, the glutamate transporter is not capable of reuptake of the neurotransmitter into the cell. Therefore, in condition of massive death of neuronal cells, the amount of the released glutamate is growing exponentially, causing excitotoxic excitation in still living neurons and leading to its death.

Glutamatergic system of the brain is one of the most widely specialized signaling systems in our...
brain and nervous system, and its role is difficult to overestimate [9].

The aim of this study is the identification and quantitative assessment of alkali-labile sites and DNA strand breaks in leukocytes of peripheral blood of male mice treated orally with Rapitalam.

Materials and methods

The method is based on evaluation of DNA integrity in whole blood leukocytes of animals and humans.

The experiment was performed on small laboratory rodents (males of mice), with an average weight of 35-40 g and 2-4 months of age. The animals were kept in accordance with the applicable Sanitary rules on the device, equipment and maintenance of experimental biological clinics in BelSU Clinical and Preclinical Studies Centre, on a standard diet, with 12-hour light mode, in conditions of free access to water and food. Obtained from the nursery animals were distributed in randomized groups of 6 individuals. As a negative control there was used animals that were injected solvent. The exposure time, the conditions of the keeping of the negative control animals and animals receiving the test substances were identical [2].

The test pharmaceutical substance of Rapitalam was dissolved in dimethyl sulfoxide to final concentration of solvent 5%. Acute dose was 413 mg/kg, which corresponds to 1/5 of LD50 dose (according to studies acute toxicity the LD50 of the test drug was 2066 mg/kg). The therapeutic dose was 3 mg/kg. All solutions and suspensions were prepared immediately before use. Rapitalam was administered to animals orally in two ways, either a single acute dose or once a day therapeutic dose for 4 days. The substance was administered orally.

For analysis we used a peripheral blood of the mice obtained by incising the tip of the tail. The level of DNA damage in peripheral blood cells of animals after administration of acute dose of DMSO and/or Rapitalam (M ± SD).

Aliquots of diluted blood were mixed with an equal volume of 1% low-melting agarous (“Sigma Chem. Co.”, USA) at a temperature of 37° C and applied to the prepared agarous layer. After hardening of the agarous, containing the cells, on top there was applied a new layer of 0.5% low-melting agarous. The slides were placed in lysing solution (2.5 mol/l NaCl, 0.1 mol/l EDTA, 0.01 mol/l Tris-HCl, pH 10, 1% Triton X-100) at 4-6°C for 1 h. Then the slides were transferred for 20 min in alkaline solution (0.3 mol/l NaON, 0.001 mol/l EDTA, pH >13), transferred to the electrophoresis chamber SE-1/5-1N (LLC “Helicon”, Russia) and subjected to electrophoresis in a fresh portion of the alkaline solution (250 ml) for 20 min at 4-6°C (voltage 27 V, current 260-270 mA, the strength of the electric field 2 V/cm).

After electrophoresis, the slides were washed with distilled water and stained for 1 h in a solution containing 2.0 µg/ml of ethydium bromide. The preparations were analyzed using a fluorescence microscope “LUMAM I-3” (“LOMO”, Saint-Petersburg, Russia). Image capture was performed with a digital camera “Nikon CoolPix 995” (Japan) with the subsequent transfer them to the computer. The processing of the photomicrographs was performed using specialized software, where there were implemented the algorithms of calculation of standard parameters of "comet" [2]. For each experimental point there was taken for 6 mice and prepared 3 slides of whole blood from each animal and photographed at 50 "comets" slide [3], that is, for each microslide there were analyzed no less than 150 DNA comets with no overdubs of tails. The analysis of parameters of DNA comets was performed with the stored digital images. As an indicator of DNA damage there was used the value of %TDNA - % DNA in the tail of the comet. Statistical analysis was performed using student’s t-test (p < 0.05). The middle values presented as M ± SD.

Research results

Data on parameters of DNA damage for each mouse, established after administration of Rapitalam and/or 5% DMSO, are shown in tables 1 and 2. Table 3 shows average values of DNA damage in groups while taking the drug and/or solvent for this drug.

<table>
<thead>
<tr>
<th>Number of the animal</th>
<th>Number of analyzed cells</th>
<th>%TDNA</th>
<th>Number of the animal</th>
<th>Number of analyzed cells</th>
<th>%TDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>12.7±1.3</td>
<td>7</td>
<td>150</td>
<td>24.5±6.5</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>17.75±8.4</td>
<td>8</td>
<td>150</td>
<td>27.37±3.5</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>21.34±5.4</td>
<td>9</td>
<td>150</td>
<td>23.63±4.0</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>19.5±2.5</td>
<td>10</td>
<td>150</td>
<td>24.51±5.3</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>15.89±3.9</td>
<td>11</td>
<td>150</td>
<td>22.07±9.1</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>11.81±8.5</td>
<td>12</td>
<td>150</td>
<td>17.39±3.8</td>
</tr>
</tbody>
</table>

Note: no significant differences between the values of %TDNA for DMSO and Rapitalam from individual animals.

The level of DNA damage in peripheral blood cells of animals after administration of acute dose of DMSO and/or Rapitalam (M ± SD).
**Research Result: Pharmacology and Clinical Pharmacology.**

**Conclusions**

1. Analysis of the level of DNA damage of blood leukocytes (%TDNA) in the groups with acute dose of Rapitalam (Table 3) showed significant differences between animals treated with the solvent from mice treated with dissolved in DMSO Rapitalam (p = 0.008). It indicates the presence of DNA-damaging activity of Rapitalam in the acute dose.

2. Analysis of the level of DNA damage of blood leukocytes (%TDNA) in groups with therapeutic dose of Rapitalam showed significant differences between animals treated with the solvent and animals treated with dissolved in DMSO Rapitalam (p = 0.0009) (see Table. 3).

**References**


**Table 2.**

The level of DNA damage in peripheral blood cells of animals after administration of the therapeutic dose of DMSO and/or Rapitalam (M ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acute dose, 1/5 of LD50</th>
<th>Therapeutic dose, 3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%TDNA</td>
<td>p</td>
</tr>
<tr>
<td>Rapitalam</td>
<td>23.25 ± 3.35</td>
<td>0.008</td>
</tr>
<tr>
<td>DMSO, 5%</td>
<td>16.51 ± 3.75</td>
<td></td>
</tr>
</tbody>
</table>

Note: differences between the values of %TDNA for DMSO and Rapitalam as in the acute dose and the therapeutic dose are significant.

* significantly different from the value for acute dose of Rapitalam (p < 0.05).

**Table 3.**

The influence of Rapitalam on the level of DNA damage in peripheral blood leukocytes of the mice (M ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acute dose, 1/5 of LD50</th>
<th>Therapeutic dose, 3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%TDNA</td>
<td>p</td>
</tr>
<tr>
<td>Rapitalam</td>
<td>1.56±0.5*</td>
<td></td>
</tr>
<tr>
<td>DMSO, 5%</td>
<td>1.22±0.6*</td>
<td></td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>1.56±0.5*</td>
<td></td>
</tr>
</tbody>
</table>

Comment: * - significantly different from the value for DMSO (p < 0.05).
UDC: 615.03+616-073.97: 615.214.32
DOI: 10.18413/2500-235X-2016-2-4-12-20

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EVALUATION EFFICIENCY OF MODERN ANTIDEPRESSANTS BY MEANS OF QUANTATIVE PHARMACO-EEG

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Abstract

Introduction. Currently, topicality the problem of diagnosis and treatment of depressive disorders (DD) in medical practice is not in doubt. The drugs of choice in the treatment of this affective disease are the selective serotonin reuptake inhibitors (SSRIs). One of the best known and widely used today of representatives a number of SSRIs is fluoxetine. An example perspective way to improve the pharmacotherapy of depression is to combine SSRIs with representatives of other groups of drugs. Great interest from this point of view is the hormone of pineal gland - melatonin (MT), which is an important element of the non-specific antistress system of body. One more potentially significant vector of perfecting therapy is searching/development of the new, more potent and fast-acting antidepressants. In this respect, the attention of researchers attracts agonist to MT₁ and MT₂ receptors and antagonist to 5-HT₂ receptors - valdoxan. Materials and methods. In this study, by means of quantitative pharmaco-EEG (QPEEG) was performed a comparative evaluation of pharmacological activity of valdoxan, fluoxetine and combination of fluoxetine with melaxen during rat's experimental depression (ED) and depressive disorder (DD) in patients. Pharmacoeconomic analysis was performed by the method of "cost/effectiveness". Results and discussion. It was established that reception of valdoxan and combined fluoxetine with melaxen promotes a more rapid normalization of bioelectric activity of brain than at fluoxetine usage separately. So, on the EEG at rats with ED was registered significant increase of theta-rhythm activity, which dominates in the norm, and at patients with DD this regime of pharmacotherapy normalized the distribution of the alpha-rhythm and reduced slow-wave activity. Conclusions. Evaluation efficiency of the above regimes of pharmacotherapy of depressive frustration showed, that the application of valdoxan, and also combination fluoxetine+melaxen favorably affects the course of the disease and contributes to a more rapid normalization of bioelectric activity of brain than fluoxetine in isolation, both experimentally and clinically. Keywords: depression, rats, patients, valdoxan, fluoxetine, melaxen, pharmaco–EEG.

Introduction. Currently, there is a distinct increase of the DD in the general population [1] and in patients with chronic somatic diseases [2]. According to the principles of evidence-based medicine group of antidepressants – selective serotonin reuptake inhibitors (SSRIs) are considered...
as standard and must be included in the plan for the treatment of affective disorders. SSRIs, as we know, have a wide range of pharmacological effects, including: anxiolytic, expressed analgetic and antipanic. Clinical efficiency of this group of antidepressants is proved at treatment of chronic pain, bulimia, obesity, alcoholism, obsessive-compulsive frustration, panic frustration etc. [3, 4].

Among the best known and widely used today of representatives a number of SSRIs reversible type of action is fluoxetine. This drug increases the extracellular concentrations of serotonin, slightly influences on norepinephrine and dopamine and almost not modulates the activity of choline- and histamine H<sub>1</sub>-receptors. This explains the absence sedative and cardiotoxic potential of this drug. If we compare fluoxetine with antidepressants of other groups (three- and heterocyclic), it can be described as the drug with a minimum of side-effects, and more secure, even at absolute overdose [5]. However, at the majority of patients, who use fluoxetine, observed anxiety, agitation, sleep disturbances, sexual dysfunction [6, 7].

One of the most perspective ways to improve the pharmacotherapy of depression is to combine SSRIs with representatives of other groups of psychotropic (and other) drugs. Thus, there is evidence on the joint use of antidepressants with antipsychotics in order to improve the treatment of psychosis and drug-resistant forms of depression [8]. But of much greater interest to the combined pharmacotherapy of depression is the hormone of pineal gland - melatonin (MT), which is an important element of the non-specific antistress system of body and control cycle "sleep-wake" [9].

But, one more potentially significant vector of perfecting of antidepressive therapy is searching/development of the new, more potent and fast-acting antidepressants including those with unique mechanisms of action. In this respect, the attention of researchers and practitioners attracts valdoxon, whose action is based on the agonism to MT<sub>1</sub>- and MT<sub>2</sub>-receptors and antagonism to 5-HT<sub>2C</sub>-receptors [10].

Purpose of the study.
The main objective of our research was to analyze changes of the indices of bioelectric activity of brain by means of QPEEG dynamics in rats with ED and in patients with ED during treatment by modern antidepressants.

Materials and methods.
The object of the experimental study were 120 white outbred male rats weighing 150-180 g (at beginning of experiment), which were divided into 5 groups (2 - control and 3 - experimental) 30 animals in each. Control groups were as follows: the intact rats (group C) (n=30) and rats, with modelled ED (group D) (n=90). Group D was subsequently divided into 3 experimental groups, in which animals during the experiment had a per os treatment by following drugs: fluoxetine in dose of 0.3 mg/kg/day (group F) (n=30); valdoxan in dose of 0.5 mg kg/day (group V) (n=30); fluoxetine - 0.3 mg/kg/day and melaxen 0.05 mg/kg/d (group F+M) (n=30). Modeling rat's ED included 3 stages: stage 1 (the 1st week) - the animals once a day were exposed to a 2-hour immobilization in a plastic container; stage 2 (the 2nd week) - once a day, for 30 minutes induced water-immersion cold stress; stage 3 (3-4 weeks) - was applied 2 above-stated stress factors. Throughout all 4 weeks of experiment the animals were also subjected to chronic exposure to light. EEG recording was made on the 7th, 14th and 21st days of receiving the drugs. According to stereotaxic coordinates to rats of all groups to the area of somatosensory cortex (SSC) and CA<sub>3</sub> region of hippocampus (HC) symmetrically on both sides microelectrodes were implanted, and after 3 days EEG was recorded. Next, the rats of experimental groups for 3 weeks of experiment had a per os treatment by studied drugs.

In a clinical study 45 people were included. Group D (n=45) consisted of patients with a diagnosis of DD moderate; group C (n=17) - practically healthy people; group F (n=15) - patients treated for 42 days fluoxetine in dose of 20 mg/day per os once in the morning after food; group V (n=15) - patients treated for 42 days valdoxan in dose of 25 mg/kg/day per os once hour before bedtime and, finally, group F+M (n=15) - patients treated for 42 days combination of fluoxetine with melaxen per os at doses of 20 mg/kg/day once in the morning after food and 3 mg/kg/day once hour before bedtime, respectively. The criteria take into account the effectiveness of pharmacotherapy EEG data in patients before prescribing medication and on the 14th, 28th and 42nd days of treatment.

Register EEG in experiment and clinical study was registered with usage 8-channel encephalograph EEGA-21/26 "Encephalan 131-03" ("Medicom-MTD", Russia), in accordance with the generally accepted rules. We analyzed the relative values of power (RVP) (%) for delta- (δ-), theta- (θ-), alpha- (α-) and beta- (β-) frequency EEG bands.

Pharmacoeconomic analysis was performed by the method of "cost/effectiveness". The outcome of this analysis was the cost-effectiveness ratio, calculated by the formula: CEA = cost of treatment (RUB.) / RVP (%) of α-rhythm in the occipital region.

Results and discussion.
During the analysis of indexes EEG rhythms in the experimental part of our work it was established that for group was characteristic significant dominance of θ-rhythm in all leads, its larger activity was noted in HC, than in SSC. δ-rhythm was
considerably less, and α- and β-rhythms were virtually not registered.

At the ED distribution of power values of EEG rhythms in rats changed in such a way that the θ-rhythm significantly decreased, and began to dominate the δ--rhythm. RVP (%) EEG rhythms of rats at reference state and at the ED are shown in Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>ELECTRODE LOCALIZATION</th>
<th>EEG RHYTHM</th>
<th>GROUPS OF ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>22,29±2,71</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>60,95±4,06</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>7,00±0,38</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>9,76±0,38</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>27,43±2,34</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>56,84±5,89</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>6,74±0,29</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>8,99±0,34</td>
</tr>
</tbody>
</table>

Note: *- at p<0.05 as compared to animals of group C; ** - at p<0.01 as compared to animals of group C.

On the 7th day in group F, where rats received fluoxetine, change of EEG rhythms consisted in reliable decrease δ- and a slight increase in θ-activity as compared to indexes at ED.

At introduction of valdoxan (in group V) changes of RVP of EEG rhythms affected mainly δ-rhythm, which decreased significantly in 2 times in the SSC and a little less - in HC, but θ-rhythm, on the contrary, progressively increased in both zones registration. β-activity has also undergone a change in the direction of increasing, exceeding the value of C in the SSC of 1.6 times.

In the study of RVP of EEG rhythms in rats in group F+M, there was a significant decrease in the level of δ- and a slight increase - θ-range compared to the ED. In addition, in both derivations recorded increase β-rhythm, which was to prevail over the performance of C is almost 2 times (Table 2).

**Table 2.**

<table>
<thead>
<tr>
<th>ELECTRODE LOCALIZATION</th>
<th>EEG RHYTHM</th>
<th>GROUPS OF ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>45,57±3,96**</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>38,05±3,14**</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>7,56±0,64</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>8,82±0,63</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>43,96±2,84**</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>40,54±3,50##</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>5,85±0,52</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>8,65±0,42</td>
</tr>
</tbody>
</table>

Note: *- at p<0.05 as compared to animals of group C; ** - at p<0.01 as compared to animals of group C; - at p<0.05 as compared to animals of group D; - at p<0.01 as compared to animals of group D.

On the 14th day of observation at rats in group F δ-rhythm progressively decreases, the θ-rhythm increased; β-activity strengthening, more expressed in the field of SSC, than in HC.

At rats to whom entered valdoxan the θ-rhythm with excess of its values in all recorded assignments (especially - in HC) in comparison with indexes of norm dominated. The δ-rhythm was below values of group C, and the β-rhythm remained increased in the field of SSC.

At introduction of a combination of MP in the F+M group the δ-rhythm decreased twice in relation to indexes at ED, a θ-rhythm, increased, but did not reach original values. Power of a β-rhythm increased more, than twice in both assignments in comparison with the indicators received and at ED, and is normal (Table 3).

The comparative characteristic of RVP (%) of EEG rhythms of rats of groups F, V and F+M on the 14th day of introduction.

<table>
<thead>
<tr>
<th>ELECTRODE LOCALIZATION</th>
<th>EEG RHYTHM</th>
<th>GROUPS OF ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td>HC</td>
<td>δ</td>
<td>36,11±2,22**##</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>42,86±3,43**##</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>6,51±0,55</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>16,20±2,36**##</td>
</tr>
<tr>
<td>SSC</td>
<td>δ</td>
<td>34,80±2,00**##</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>39,87±2,33**</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>6,26±0,37</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>20,79±2,51**##</td>
</tr>
</tbody>
</table>

Note: * - at p<0.05 as compared to animals of group C; ** - at p<0.01 as compared to animals of group C; * - at p<0.05 as compared to animals of group D; ## - at p<0.01 as compared to animals of group D.

The analysis of the EEG parameters at animals in group F for the 21st day of a pharmacotherapy of fluoxetine showed that the θ-rhythm was synchronized and had the exact distribution, but values of its RVP were authentically below norm. In the field of group of companies they decreased in comparison with indexes at ED by 1,5 (р<0,01), and in the field of SSC by 1,2 (р<0,05). Indexes of RVP of a δ-rhythm did not reach norm and remained increased: in assignment of group of companies - by 1,6 times, and in SSC - by 1,2 times, at the same time having decreased concerning indexes at ED both in group of companies, and in SSC by 1,5 (р<0,01). RVP of a β-rhythm in the field of HC obviously increased in 2 (р<0,01), and in SSC - by 3 (р<0,01) in comparison with group D, and on comparison by group C. The α-rhythm remained invariable, and corresponded to values of norm (figure 1).

At the rats receiving valdoxan, RVP values and θ- and δ-rhythms were restored in the conditions of ED much quicker. Low indicators of δ-activity in comparison with monitoring in 1,4 - 1,9 (р<0,05) in both assignments were noted. The θ-rhythm exceeded input datas both in the field of SSC, and in HC for 16% (р<0,05). Recorded increase in twice RVP of β-rhythm in SSC (р<0,01) (figure 2).

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Figure 2. Distribution of RVP of EEG rhythms of rats for the 21st day of introduction of valdoxan.

At animals of the F+M group indexes were similar with group F, except minor changes of activity of a β-rhythm which increased twice (p<0,01) in comparison with norm more narrow for the 7th day, and a δ-rhythm which later from the beginning of introduction the combination of drugs corresponded 14 days to monitoring in the field of SSC, but still remained increased by 1,4 times (p<0,01) in the field of HC (figure 3).

Figure 3. Distribution of RVP of EEG rhythms of rats for the 21st day of introduction of combination of fluoxetine with melaxen.

During the clinical study we defined - how rhythmic activity of the brain at patients with DD of moderate severity and how its parameters are influenced by the carried-out pharmacotherapy antidepressants changes.

In the beginning filing of an EEG at healthy people of group C and patients with DD was carried out (group D). In group C the α-rhythm which was registered in an occipital lead and decreased towards the frontal departments of a brain dominated. The β-rhythm was fixed in the frontal area with gradual decrease to a nape, and δ- and θ-rhythms were a little expressed in all assignments.

When filing an EEG at patients with DD distribution of rhythms significantly changed. The dominating place was taken now by a δ-rhythm, increase in RVP of a θ-rhythm was also noted, and the maximal values of a α-rhythm recorded to the area of a forehead, and in all recorded assignments decrease of RVP of a β-rhythm was observed (table 4).

Table 4. The comparative characteristic of RVP (%) of EEG rhythms at healthy people and patients with a DD.

<table>
<thead>
<tr>
<th>ELECTRODE LOCALIZATION</th>
<th>EEG RHYTHM</th>
<th>GROUPS OF PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Occipital area</td>
<td>δ</td>
<td>9,28±2,94</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>3,47±1,55</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>76,24±5,55</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>11,65±4,27</td>
</tr>
<tr>
<td>Parietal area</td>
<td>δ</td>
<td>7,36±2,53</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>6,17±1,48</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>59,12±6,87</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>26,88±2,32</td>
</tr>
<tr>
<td>Central area</td>
<td>δ</td>
<td>5,20±2,67</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>4,54±1,60</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>33,06±5,38</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>57,88±7,49</td>
</tr>
<tr>
<td>Frontal area</td>
<td>δ</td>
<td>6,21±2,61</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>4,09±1,47</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>25,28±3,85</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>64,90±7,90</td>
</tr>
</tbody>
</table>

Note: * - at p<0.05 as compared to group C; ** - at p<0.01 as compared to group C.

At the pharmacotherapy of fluoxetine change of EEG rhythms concerned α-rhythm, which began to dominate in occipital area for the 28th day of treatment and was higher in 2.8 (p<0.01) than indexes at a depression. For the 42nd day of a pharmacotherapy of its RVP remained below input datas in 1.3 (p<0.05). The β-rhythm was distributed correctly too, but slightly laged behind norm in the frontal and central departments for 28% (p<0.05), the δ-rhythm kept authentically high rates of RVP in all recorded assignments (p<0.01), and a θ-rhythm, having reached background values for the 28th day, remained invariable until the end of observation (figure 4).

Figure 4. Distribution of RVP of EEG rhythms at patients for the 42nd day of introduction of fluoxetine.

Note to figures 4-6: on an axis of ordinates – RVP (%), on an abscissa axis – the field of registration of EEG rhythms; * at p<0.05 as compared to group C; ** - at p<0.01 as compared to group C; * - at p<0.05 as compared to group D; ** - at p<0.01 as compared to group D

Reception of a combination of F+M affected on a rhythmicity, almost similar to that one F. At the 42nd day of a pharmacotherapy α-and β-rhythms did not reach initial indicators, and the δ-rhythm remained increased ≈ 2.5 - 3 in all assignments in comparison with values in group C (figure 5).
The analysis of RVP of an EEG rhythms at the patients accepting valdoxan showed that for the 28th day the α-rhythm began to correspond to values in group C, except for occipital area where indicators of its RVP increased only for the 42nd days of a pharmacotherapy and exceeded input datas for 14.4% (p<0.05). The β-rhythm was not exposed to special modulations unlike a δ-rhythm which underwent reliable regress: in occipital, parietal and central area-by 4,2–3,4 times (p<0.01), and in the frontal area - by 5,1 times (p<0.01), having also reached the group C RVP level in all assignments, and for the 42nd days corresponded to input datas. The θ-rhythm reached values of norm more narrow for the 14th day. On the person the fact that patients with DD of moderate severity have an increase and normalization of values of fast-wave activity (α- and β-rhythms) in the respective areas of filing of an EEG against the background of reception In occurs quicker, than against the background of F including at a combination of F+M (figure 6).
At finally, the pharmacoeconomic analysis of 2 schemes treatment of DD of moderate severity (valdoxan and a combination fluoxetine + melaxen) by means of the expense/effectiveness method was carried out.

As criterion of effectiveness we accepted a difference (Δ) of RVP values (%) of α-rhythm in occipital area of patients for the 28th and 42nd days of observation in comparison with its indexes at a depression. Data on dynamics of it of an EEG index in groups of comparison are provided in table 5.

Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation time</th>
<th>Group V</th>
<th>Group F+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVP (%) of α-rhythm in the occipital area of the brain</td>
<td>Norm</td>
<td>76,24±5,55</td>
<td>20,70±2,00*</td>
</tr>
<tr>
<td></td>
<td>Depression</td>
<td></td>
<td>58,86±6,57*</td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>60,81±8,95</td>
<td>40,1±6,2*</td>
</tr>
<tr>
<td></td>
<td>Δ by 28 days</td>
<td>90,29±4,56*</td>
<td>38,3±4,3*</td>
</tr>
<tr>
<td></td>
<td>42 days</td>
<td>90,29±4,56*</td>
<td>58,28±5,05*#</td>
</tr>
<tr>
<td></td>
<td>Δ by 42 days</td>
<td>69,6±5,3*</td>
<td>37,6±3,9*#</td>
</tr>
</tbody>
</table>

Note: * - at p<0.05 as compared to original value in this group; # - at p<0.05 as compared to similar value in group V.

Following the results of processing of results it is possible to conclude that the ratio of cost 28 a day course of treatment In to its influence on an indicator of RVP of a α-rhythm exceeds F+M combination, that at application, by 2,2, at the same time this rhythm in both groups does not reach even original values. However, for the 42nd days of a pharmacotherapy this difference became not the so considerable and in group V exceeded the corresponding indicator of the F+M group for 32,5% (i.e. was most of all by 1,4) (figure 7).

![Figure 7. Cost-effectiveness of pharmacotherapy course at patients surveyed groups on RVP of a α-rhythm of the brain.](image-url)

Note: on an axis of ordinates – the drug cost per course of treatment (rub.) / RVP (%) α-rhythm on the 28th and 42th days of observation. On an abscissa axis – the period of observation.

Summary.

According to results of the clinical and experimental study of changes in the bioelectric activity of the brain by means of QPEEG against the background of antidepressant use can be concluded that the low values of θ-rhythm, and also increase δ- and β-activities of brain groups of rats, who received fluoxetine separately or in a combination with melaxen are bound, probably to the presence of this antidepressant anxiogenic effects. The combination of F+M has a similar effect on EEG rhythms in rats with the only difference that the enhancement of β-activity was observed on the first week of introduction of the studied drugs. This, in our opinion, may be associated with the ability melaxen has modulatory effects on subcortical structures of the brain [11].

In turn, valdoxan, not only has a modulating effect on θ-activity detected in all leads, but also reduces the δ-rate increase and β-rate, especially in the SSC. It can be assumed that such changes are associated with a unique mechanism of action of valdoxan, which distinguishes it from other...
antidepressants, and, above all, its activating effect on MT$_1$-and MT$_2$-melatonin receptors.

The analysis of RVP of EEG rhythms at patients showed that the main role in modulation of rhythmic activity of a brain at patients of groups F and F+M is played by fluoxetine which, though promotes the exact distribution of a-rythm, nevertheless does not allow to be restored to it fully. The same can be told also about ß-activity. This drug also promotes preservation of the increased ð-rythm power in all recorded assignments, that correlates with conclusions of other authors [12, 13].

Based on the above it can be concluded that at pharmacotherapy at valdoxan the rhythmic activity of a brain is characterized by much more rapid positive dynamics of indexes of an EEG from all carried-out treatment options that is explained by the unique mechanism of action of this drug and, in particular, its melatonin-mimetic potential. It is important that unlike the tricyclic antidepressants, SSIRs and inhibitors of MAO valdoxan, according to literature, does not influence serotonin level in the brain [14]. Some importance in realizing the effects of the arrangements may also have antagonism in relation to 5-HT2c-receptors of serotonin and increased catecholamine (dopamine and norepinephrine) in the frontal cerebral cortex [15].

Thus, summarizing the received results, we can say that the application of a valdoxan, and also combination fluoxetine+melaxen at treatment of depressive frustration of moderate severity, favorably affects the course of the disease and contributes to a more rapid normalization of bioelectric activity of brain than fluoxetine in isolation.

**Conclusions.**

1. Long-term usage of valdoxan in contrast to the fluoxetine and combination of fluoxetine with melaxen helps to normalize the distribution of EEG rhythms in rats with ED, as evidenced by an increase in RVP of ð-rythm of 16% in the HC and SSC and increase in RVP of ß-rythm of 2 times in the SSC.

2. QEEG comparative analysis of the results shows that the most favorable effect on the rhythmic brain activity in patients with DD as compared to other circuits has valdoxan therapy, what confirmed, first of all, in the increase of 2.9 times (as compared to the depression) and normalization RVP of ß-rythm EEG already on the 28th day of treatment.

3. By means of pharmacoeconomic analysis was showed that as compared to combination fluoxetine+melaxen for usage valdoxan characterized more favorable values of the coefficient "cost/effectiveness" exceeding those of fluoxetine with melaxen by 32.5% in terms of impact on the bioelectric activity of the brain at patients with depression.

**References**


Larin S.L., Zvyagintseva A.R., Habarov A.A., Budko E.V.

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

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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 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 intraperitoneal injection 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 "Soyuzsvetmetavtomatika" 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/dm³.

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 Zn²⁺ 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.

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.

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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<sub>0-t</sub> (h×μg/ml), AUMC (h<sup>2</sup>×μg/ml), Cl<sub>T</sub> (ml/h), MRT (hour), C<sub>max</sub> (μg/ml), t<sub>max</sub> (h), T<sub>1/2</sub> (h), and C<sub>max</sub>/AUC<sub>0-t</sub> (1/hour), f<sub>a</sub>(%). 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 ± m; n=3 (per group)).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Single enteral administration of nanoform of zinc hydroxide.</th>
<th>Single enteral administration of the dissolved zinc sulphate.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dosage (mg/kg)</td>
<td>Dosage (mg/kg)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;, h×μg/ml</td>
<td>1040.47 ± 40.85</td>
<td>3580.90 ± 60.86</td>
</tr>
<tr>
<td>AUMC, h&lt;sup&gt;2&lt;/sup&gt;×μg/ml</td>
<td>1243.78 ± 39.36</td>
<td>3793.56 ± 77.23</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;T&lt;/sub&gt;, ml/h</td>
<td>9.60 ± 0.38</td>
<td>13.96 ± 0.24</td>
</tr>
<tr>
<td>MRT, h</td>
<td>1.19 ± 0.01</td>
<td>1.06 ± 0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>120.73 ± 6.47</td>
<td>390.58 ± 10.38</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;, h</td>
<td>1040.47 ± 40.85</td>
<td>3580.90 ± 60.86</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;/AUC&lt;sub&gt;0-t&lt;/sub&gt;, h</td>
<td>0.12 ± 0.01</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>F&lt;sub&gt;a&lt;/sub&gt;, %</td>
<td>30.33 ± 3.16</td>
<td>44.39 ± 4.52</td>
</tr>
</tbody>
</table>
The maximum concentration of $\text{Zn}^{2+}$ in blood plasma after administration of compounds has the following trends: at a dose of 10 mg/kg $C_{\text{max}}$ (p<0.05) higher for zinc sulfate than for nanoparticles of zinc hydroxide (169.72±7.63 μg/ml and 120.73±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 – $t_{\text{max}}$ 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$_{\text{a}}$) 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Single injection of nanoforms of zinc hydroxide (M ± m; n=3 (per group))</th>
<th>Single injection of sulfate zinc. (M ± m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dosage (mg/kg)</td>
<td>AUC, h× µg/ml</td>
</tr>
<tr>
<td>10</td>
<td>34.13 ± 2.67</td>
<td>59.17 ± 1.52</td>
</tr>
<tr>
<td>50</td>
<td>80.02 ± 9.45</td>
<td>114.26 ± 5.89</td>
</tr>
<tr>
<td>100</td>
<td>150.72 ± 11.86</td>
<td>150.72 ± 11.86</td>
</tr>
<tr>
<td></td>
<td>38.00 ± 2.48</td>
<td>64.59 ± 4.74</td>
</tr>
<tr>
<td></td>
<td>82.13 ± 9.79</td>
<td>131.01 ± 10.31</td>
</tr>
<tr>
<td></td>
<td>153.29 ± 10.13</td>
<td>257.01 ± 14.79</td>
</tr>
</tbody>
</table>

For all groups the maximum concentration of zinc ions Zn²⁺ 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 ± 24.45 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 Zn²⁺ 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 Zn²⁺ (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.

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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 ZnSO₄ has the statistical significance (p<0.05) and differed among each of the groups, decreasing in the series Zn(OH)₂ – ZnO – ZnSO₄. 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.

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).
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.

- **A**: Control group Zn$^{2+}$ level 3.67 μg/g
- **B**: Control group Zn$^{2+}$ level 3.67 μg/g
- **C**: Control group Zn$^{2+}$ level 3.67 μg/g

Time in the experiment, h

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.**


Hypertension, existing for a long time, leads to serious complications in the so-called target organs (kidney, heart, brain, retina). Changes in the eye of high blood pressure among other manifestations of this pathology have a special place. The picture of the fundus and indicators of local hemodynamics significantly complement the representation of researchers about the features of the disease, can detect early signs of organic changes in the retinal vessels and judge by their state with a certain degree of confidence about the changes of regional vascular bed and on the vascular system of the body as a whole [1].

63% of hypertensive patients have evidence of hypertensive angiopathy [2]. To assess the changes in the fundus caused by hypertension, used classification of Krasnov ML, according to which there are three steps: stage I – hypertonic angiopathy; stage II – a hypertonic angiosclerosis; stage III – hypertensive angioretinopathy and neuroretinopathy. Hyper-tensive angiopathy is characterized by the first phase of hypertension, in which there are only functional vascular disorders and the pressure is unstable.

Speaking about the pathogenesis of hypertensive retinopathy, it should be noted three main factors: the restriction and increased vascular permeability, and arteriosclerosis. Expressed arteriolar narrowing due to spasm of the walls takes place in response to increased blood pressure. The presence of bleeding on the surface of the retina caused by ruptures of the capillaries in the retinal nerve fiber layer in the posterior pole of the eye area [3].

The above changes in the retina, of course, lead to disruption of blood supply and the development of ischemic conditions, which can be corrected by pharmacological preconditioning. The phenomenon of ischemic preconditioning eventually realized by activation of the ATP-dependent potassium channels. As an end-effector of preconditioning, the ATP-dependent potassium channels cause hyperpolarization of the cell membrane, as well as launching a system of nitrous oxide and a number of anti-apoptotic mechanisms [4, 5, 6, 7].

From our point of view, one of the most promising drugs with the effect of pharmacological
preconditioning and unstudied as retinoprotectors are minoxidil and sildenafil.

The cardioprotective effect of minoxidil during heart preconditioning proved in experimental researches in vivo and in vitro [8]. The use of minoxidil (0.5 mg/kg/day) during simulated ischemia of the lower leg muscle helps to increase the level of the microcirculation to the 28th day in 2.3 times in comparison with the control group, levels ischemic damage muscle tissue [9].

Recent studies have shown that inhibitors of phosphodiesterase - 5, in particular, sildenafil, have a stimulating effect on the nitric oxide pathway, exerting pronounced endothelioprotective effect, capable of activating protein kinase G and the ATP-dependent potassium channels [10, 11].

In connection with the above, it should be noted the relevance of study of retinoprotective effects of minoxidil, sildenafil on model of retinal angiopathy of hypertensive type.

Objective: to increase the effectiveness of pharmacological correction of retinal angiopathy of hypertensive type using pharmacological preconditioning by minoxidil, sildenafil.

Materials and methods.

Experiments were carried out on Wistar rats weighing 225-275 g. The rats were taken for the study with no external signs of disease, passed quarantine regime.

Operations and other manipulations were performed on rats under general anesthesia by intraperitoneal (i/p) introducing an aqueous solution of chloral hydrate in a dose 300 mg/kg.

L-NAME was injected in a dose 12.5 mg/kg/day for 28 days i/p daily.

DIP was performed 10 min by the clamping the femoral artery to the proximal tourniquet third thigh for 40 min before the administration of L-NAME in odd days of the experiment.

Minoxidil was administered intragastrically (i/g) in a dose 0.5 mg/kg 60 min before the administration of L-NAME in odd days of the experiment (every 48 hours). This dose of minoxidil is chosen by us, based on the literature data on pharmacological preconditioning by minoxidil [8, 9]. Drug administration explained by possible reduce of expressed hypotensive action due to the short half-life (4 hours) and longer period (48 hours) of preconditioning action [12].

Sildenafil was administered i/p in a dose 0.5 mg/kg 30 min before the administration of L-NAME in odd days of the experiment. This dose is chosen by us, based on the literature review of study of a possible correction of ischemic-reperfusion injury of various organs and tissues by sildenafil [13, 14, 15].

Glibenclamide, a blocker of ATP-sensitive potassium channels, was administered i/g in a dose 5 mg/kg [16] 90 min before the administration of L-NAME in odd days of the experiment.

To measure blood pressure in rats (tail) a system of non-invasive measurement of blood pressure for small animals NIBP200 was used in the complex Biopac-systems MP-150.

To investigate the fundus of experimental animals a direct ophthalmoscopy was used on 29 day of the experiment (ophthalmoscope Bx a Neitz, Japan). To expand the pupil the eye drops Irifrin 2.5% were used. Ophthalmoscope has been approached to the rat eye and we sent in it a beam of light from a distance of 0.5-2 cm to obtain a clear picture of the fundus image. In the dim image of the fundus we picked up the lens by turning the disc of ophthalmoscope, which gives crisp image details of the fundus. To zoom a lens Osher MaxField 78D model OI-78M has been used.

For subsequent statistical processing the degree of change, detected during ophthalmoscopy, were ranking (Tab. 1).

Table 1.

Methods of integrated semiquantitative evaluation of the fundus changes, detected during ophthalmoscopy (in grades).

A set of attributes of fundus changes | Grades
---|---
Optic disc is circular or oval shape and stands out from the fundus in pale - pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. From the middle of the optic nerve exit the central vessels of the retina. Blood vessels of the retina don’t have Anastomoses. The veins and arteries are straightforward, caliber is uniform, not crimped. The general background is pink. | 0
Angiopathy. Symptom Salus-Hun I. It is characterized by the presence of sclerosis of retinal vessels in fundus and "phenomenon of chiasm", which occurs due to indentation of artery at the site of chiasm with extended vein. Expansion of vein on both sides of the chiasm. Symptom Guist - expansion and corkscrew curl of venules located around the macular; observed in hypertensive disease. | 1
Angiosclerosis. Symptom of copper wire - yellow glow of the retinal arteries; sign of hypertensive retinal angiopathy. Symptom Salus-Hun II - the formation of bulges in arteries and veins chiasm. Symptom Salus-Hun III - the disappearance of the vein at the site of crossing due to the formation of the arcuate bend, sinking deep into the retinal tissue. Symptom of silver wire. Increased vascular permeability. | 2
Retinopathy. “Cotton” exudates. Hemorrhages. In the macular area may be deposits of hard exudates in a star shape. | 3
Hypertensive neuroretinopathy. Severe discoloration of the optic nerve. Swelling of the optic disc and peripapillary retina. Multiple foci of hemorrhage and "cotton" exudate, indicates the growing ischemia. | 4

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Measuring of the microcirculation level in the rats retina was performed by LDF after examining the fundus. Registration is carried out by means of hardware and software Biopac-systems MP-150 and the needle-type sensor TSD-144 (USA) with AcqKnowledge 4.2 program. After animal anesthesia assessment of microcirculation level was carried out in ten points on the circumference of the eyeball, the recording duration of the microcirculation level readings at one point was 20 seconds. From the microcirculation level results at every point the average value has been calculated, which was taken as the indicator of the microcirculation level in the retina of the experimental animal. Value of microcirculation in the animal group was calculated as the average of the values obtained from each experimental animal in group [17].

ERG was performed immediately after the registration of the microcirculation level. For this the animals were kept in the dark for 30 minutes [18], further the animals were anesthetized (chloral hydrate, 300 mg/kg, i/p) and fixed on the table, isolated from the electromagnetic radiation. Corneal silver electrode was placed on the cornea that has been soaked by saline solution for better contact, the reference needle electrode EL452 has been placed subcutaneously in the region of the skull, ground needle electrode EL450 has been placed subcutaneously in the base of the tail. Strobe flash of white light that is connected to the stimulator STM200 by company Biopac System, Inc. (USA) has been placed behind the back of the animal, ERG registration was carried out in response to a single stimulation. Evoked biopotentials were run at a frequency of 1-1000 Hz, amplified, averaged and presented graphically on the screen using the Biopac-systems MP-150 with a computer program AcqKnowledge 4.2 (USA). ERG-recording was carried out for 0.5 seconds in each rat in groups. To assess the degree of functional damage to the retina we evaluated the ratio of amplitudes of a- and b-wave of ERG - the coefficient b/a [19]. From ten values in each group were taken the average, which was added to the protocol.

For all data the descriptive statistics were used: data are checked for normal distribution. Distribution type was determined by using the criterion of Shapiro-Wilk. In case of normal distribution the average value (M) and standard error of the mean (m) were calculated. In cases of abnormal distribution the median (Me) and the quartile range (QR) were calculated. Between-group differences were analyzed by parametric (t-Student criterion) or non-parametric (Mann-Whitney test) methods, depending on the type of distribution. Differences were determined at 0.05 significance level. Statistical analyzes were performed by using Statistica 10.0 software.

**The main part:**

The first step in study of the retinoprotective properties of pharmacological agents is the development of model of retinal angiopathy of hypertensive type.

We propose a model of retinal angiopathy of hypertensive type, which pathogenesis is associated with the development of hypertension in rats on the background of daily i/p administration of L-NAME in a dose 12.5 mg/kg/day for 28 days (SBP 204.8 mmHg, DBP 164.2 mmHg) in a group with pathology; SBP 139.2 mmHg, DBP 104.2 mmHg in the intact group, p<0.05). The confirmation of the formation of vascular changes in hypertensive type in the retina were results of ophthalmoscopy, LDF and ERG.

The study of retinoprotective action of minoxidil, sildenafil compared to DIP on model of retinal angiopathy of hypertensive type in experiment includes the following groups:

- The first (n = 10) - the group of intact animals,
- the second (n = 10) - the group with the modeling of retinal angiopathy (control),
- the third (n = 10) - with the correction of pathology by minoxidil,
- the fourth (n = 10) - with the correction of pathology by sildenafil,
- the fifth (n = 10) - with the correction of pathology by DIP,
- the sixth (n = 10) – with the introduction of glibenclamide and modeling of retinal angiopathy,
- the seventh (n = 10) - with the introduction of glibenclamide and correction of pathology by minoxidil,
- the eighth (n = 10) – with the introduction of glibenclamide and correction of pathology by sildenafil,
- the ninth (n = 10) - with the introduction of glibenclamide and correction of pathology by DIP.

**Results.**

We have developed a model of retinal angiopathy of hypertensive type in Wistar rats on the background of daily i/p administration of L-NAME in a dose 12.5 mg/kg/day for 28 days.

In accordance with the protocol the anesthesia of animals was carried out (chloral hydrate solution i/p 300 mg/kg) on 29 day of the experiment. Then were performed: ophthalmoscopy, assess the level of microcirculation in the retina by LDF, retinal electrophysiological status by ERG.

Example of ophthalmoscopy on intact animal is shown in fig. 1.

Example of ophthalmoscopy on animal with retinal angiopathy of hypertensive type shown in fig. 2.

**Figure 1.** Example of ophthalmoscopy on intact Wistar rat. Optic disc is circular or oval shape and stands out from the fundus in pale-pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. From the middle of the optic nerve exit the central vessels of the retina. Blood vessels of the retina don’t have anastomoses. The veins and arteries are straightforward, caliber is uniform, not crimped. The general background is pink.

**Figure 2.** Example of ophthalmoscopy on Wistar rat with retinal angiopathy of hypertensive type. Optic disc is circular or oval shape and stands out from the fundus in pink. The boundaries of the optic nerve disc are clear. Veins are congested, full-blooded, crimped at the periphery. Arteries are narrowed, slightly crimped. Retina is palely (ischemic). No hemorrhage. Symptom Salus-Hun I-III (arrows show).

Thus, the results of fundus research during ophthalmoscopy in experimental animals have found that the modeling of retinal angiopathy of hypertensive type with administration of L-NAME for 28 days leads to a pronounced vascular changes in the retina and signs of ischemic damage. Integral evaluation showed, respectively, 0 and 3 points for intact rats and for rats with retinal angiopathy of hypertensive type (tab. 2).

**Table 2.**

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Integral assessment in scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>0</td>
</tr>
<tr>
<td>With the modeling of retinal angiopathy of hypertensive type</td>
<td>3*</td>
</tr>
</tbody>
</table>

Note. * - p<0.05 compared with the group of intact animals.
The microcirculation level in the retina of intact animals was 743.6 ± 20.9 p.u. After pathology simulation in animal group the microcirculation level on 29 day of experiment was 431.4 ± 13.8 p.u., which is less than in the group of intact animals by 42%. LDF data obtained in the control group was significantly different from that of the group of intact animals (p<0.05) and confirm the formation of ischemia during the modeling of retinal angiopathy of hypertensive type.

Violations in hemodynamics led to changes inherent to retinal angiopathy of hypertensive type, which is also confirmed by the results of electrophysiological studies of retinal conditions. To assess the severity of the functional changes in the retina we used the ratio b-wave amplitude to the amplitude of a wave of the ERG - the coefficient b/a [19].

In the experimental evaluations of electrophysiological condition of rat retina it has been found that the ratio b/a in the group of intact animals was 2.6 ± 0.07 r.u., in the group with pathology simulation this index was significantly different from the values in the group of intact animals and was 2.2 ± 0.09 r.u. (p<0.05), lower than in the group of intact animals by 15%.

The data obtained allow us to conclude that the modeling of retinal angiopathy of hypertensive type causes a disturbance of electrophysiological state of inner retinal layers, which is characterized by decrease in the electrophysiological activity of the bipolar cells, Muller cells, amacrine and horizontal cells, due to violations of the retinal blood flow and the ischemia formation.

Data obtained during ophthalmoscopy, an integrated evaluation of the fundus changes, LDF and ERG in intact and control groups confirm the adequacy of the proposed model of retinal angiopathy of hypertensive type on Wistar rats for further research of retinoprotective properties of pharmacological agents.

This model is characterized by:

- severe vascular changes of hypertensive type in the retina and attributes of ischemic injury (3 points, p<0.05 compared with the group of intact animals) during ophthalmoscopy and integral evaluation of the fundus changes;
- statistically significant difference between values of the microcirculation level in the retina of rats in control group from values in the intact group on day 29 of the experiment after pathology modeling;
- significant reduction of coefficient b/a of electroretinogram after the pathology simulation on 29 day of the experiment in comparison with the value in the group of intact animals.

Further the study of retinoprotective action of minoxidil, sildenafil on the model of retinal angiopathy of hypertensive type compared to distant ischemic preconditioning and proof implementation of pharmacological effect through the participation of the ATP-dependent potassium channels were carried out.

On day 29 of the experiment anesthesia of animals was performed (chloral hydrate solution i/p 300 mg/kg). Further ophthalmoscopy were performed in groups of experimental animals. The experiment included 90 Wistar rats.

Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by minoxidil is shown in fig. 3.

Figure 3. Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by minoxidil in a dose of 0.5 mg/kg on day 29 of the experiment. Optic disc is circular or oval shape and stands out from the fundus in pale-pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. Blood vessels of the retina don’t have anastomoses. The veins and arteries are straightforward, no crimping. Slightly dilated veins at the periphery. The general background is pink, not ischemic.
Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by sildenafil is shown in fig. 4.

*Figure 4.* Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by sildenafil in a dose 0.5 mg/kg on day 29 of the experiment. Optic disc is circular or oval in shape and stands out from the fundus in pale pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. There is a slight vasoconstriction, "the phenomenon of chiasm", a symptom Salus-Hun I (arrow). Veins are crimped at the periphery. The background is slightly pally.

Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by DIP is shown in fig. 5.

*Figure 5.* Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by distant ischemic preconditioning on the 29 day of the experiment. Optic disc is circular or oval shape and stands out from the fundus of the eye in pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. There is a slight vasoconstriction, "the phenomenon of chiasm", a symptom Salus-Hun I, II (arrows show). Expansion of veins on both sides of the chiasm. Veins are crimped at the periphery. The background is slightly pally.
Integral assessment of fundus changes in experimental groups with the correction of pathology, detected during ophthalmoscopy, is presented in the table.

Table 3.

Influence of minoxidil, sildenafil and DIP on the complex fundus changes, found during ophthalmoscopy, on model of retinal angiopathy of hypertensive type (n = 10).

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Integral assessment in scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>0</td>
</tr>
<tr>
<td>With the modeling of retinal angiopathy of hypertensive type (control)</td>
<td>3*</td>
</tr>
<tr>
<td>Control + minoxidil, 0.5 mg/kg</td>
<td>0-1</td>
</tr>
<tr>
<td>Control + sildenafil, 0.5 mg/kg</td>
<td>1</td>
</tr>
<tr>
<td>Control + DIP</td>
<td>2*</td>
</tr>
<tr>
<td>Control + glibenclamide, 5 mg/kg</td>
<td>3*</td>
</tr>
<tr>
<td>Control + minoxidil, 0.5 mg/kg + glibenclamide, 5 mg/kg</td>
<td>2.3*</td>
</tr>
<tr>
<td>Control + sildenafil, 0.5 mg/kg + glibenclamide, 5 mg/kg</td>
<td>3*</td>
</tr>
<tr>
<td>Control + DIP + glibenclamide, 5 mg/kg</td>
<td>3*</td>
</tr>
</tbody>
</table>

Note. * - p<0.05 compared with the group of intact animals

Thus, the results of research of fundus during ophthalmoscopy and integral evaluation of the fundus changes in animal groups with the correction by minoxidil, sildenafil, DIP found pronounced retinoprotective effect of minoxidil in a dose 0.5 mg/kg, exceeding sildenafil in a dose 0.5 mg/kg and DIP consisting in reducing of ischemic injury to the retina and vascular changes of hypertensive type, which were observed in the control group. Integral evaluation showed, respectively, 0-1, 1 and 2 points for the groups of animals with the correction of minoxidil, sildenafil and DIP.

Introduction of glibenclamide in a dose 5 mg/kg 90 minutes before the administration of L-NAME in odd days of the experiment resulted in complete elimination of the positive effects of the correction of retinal angiopathy of hypertensive type by minoxidil, sildenafil, DIP, which confirms the implementation of protective effects due to the participation of ATP-sensitive potassium channels.

Assessment of the microcirculation level in the retina in experimental groups was performed by the LDF on 29 day of the experiment after ophthalmoscopy. The results are shown in table. 4.

The microcirculation level in the retina of intact rats was 743.6 ± 20.9 p.u. After the modeling of pathology in group of animals on day 29 of the experiment the level of microcirculation was 431.4 ± 13.8 p.u., which was significantly different from the values in the group of intact animals (p<0.05). In group with correction of pathology by minoxidil the microcirculation level in the retina was 730.5 ± 15.9 p.u., which was significantly different from the values in the control group (p<0.05), and tends to the value in the group of intact animals. Against the background of the correction of pathology by sildenafil the level of microcirculation in the retina was 605.1 ± 19.8 p.u, which was significantly different from the value in the control group (p<0.05) and the value in the group of intact animals (p<0.05).

Against the background of the correction of retinal angiopathy of hypertensive type by DIP the level of microcirculation in the retina was 510.8 ± 16.5 p.u., which was significantly different from the value in the group with pathology (p<0.05) and the value in the group of intact rats (p<0.05).

With the administration of glibenclamide i/g in a dose 5 mg/kg the improving of blood flow in the retina were not observed in any of the experimental groups with the correction of pathology - microcirculatory level values were not significantly different from the value in the control group. This fact confirms the implementation of protective effects of minoxidil, sildenafil and DIP through the participation of ATP-dependent potassium channels.

Table 4.

Level of microcirculation in rat retina on day 29 of the experiment (M ± m; n = 10), p.u.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Level of microcirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>743.6±20.9</td>
</tr>
<tr>
<td>With the modeling of retinal angiopathy of hypertensive type (control)</td>
<td>431.4±13.8*</td>
</tr>
<tr>
<td>Control + minoxidil, 0.5 mg/kg</td>
<td>730.5±15.9*</td>
</tr>
<tr>
<td>Control + sildenafil, 0.5 mg/kg</td>
<td>605.1±19.8*</td>
</tr>
<tr>
<td>Control + DIP</td>
<td>510.8±16.5*</td>
</tr>
<tr>
<td>Control + glibenclamide, 5 mg/kg</td>
<td>439.4±14.5*</td>
</tr>
<tr>
<td>Control + minoxidil, 0.5 mg/kg + glibenclamide, 5 mg/kg</td>
<td>436.2±12.9*</td>
</tr>
<tr>
<td>Control + sildenafil, 0.5 mg/kg + glibenclamide, 5 mg/kg</td>
<td>441.4±15.8*</td>
</tr>
<tr>
<td>Control + DIP + glibenclamide, 5 mg/kg</td>
<td>430.6±13.2*</td>
</tr>
</tbody>
</table>

Note. * - p<0.05 compared with the group of intact animals; y - p<0.05 compared with the control group.
ERG on evoked potential was performed after the measuring of the microcirculation level in the retina. The data obtained are presented in table 5.

### Table 5.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>(b/a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>2.6±0.07*</td>
</tr>
<tr>
<td>With the modeling of retinal angiopathy of hypertensive type (control)</td>
<td>2.2±0.09*</td>
</tr>
<tr>
<td>Control + minoxidil, 0.5 mg/kg</td>
<td>2.5±0.09*</td>
</tr>
<tr>
<td>Control + sildenafil, 0.5 mg/kg</td>
<td>2.3±0.08*</td>
</tr>
<tr>
<td>Control + DIP</td>
<td>2.3±0.10*</td>
</tr>
<tr>
<td>Control + glibenclamide, 5 mg/kg</td>
<td>2.2±0.08*</td>
</tr>
<tr>
<td>Control + minoxidil, 0.5 mg/kg + glibenclamide, 5 mg/kg</td>
<td>2.3±0.09*</td>
</tr>
<tr>
<td>Control + sildenafil, 0.5 mg/kg + glibenclamide, 5 mg/kg</td>
<td>2.2±0.05*</td>
</tr>
<tr>
<td>Control + DIP + glibenclamide, 5 mg/kg</td>
<td>2.2±0.09*</td>
</tr>
</tbody>
</table>

Note. * - p<0.05 compared with the group of intact animals; y - p<0.05 compared with the control group

The coefficient b/a in control group was 2.2 ± 0.09 r.u., which was significantly different from the value in the group of intact animals. Increase of this indicator in group with the correction by minoxidil up to 2.5 ± 0.09 r.u. says about the preservation of retinal electrophysiological function after disease modeling. In groups of animals with the correction by sildenafil and DIP ratio b/a was 2.3 ± 0.08 r.u. and 2.3 ± 0.10 r.u. accordingly, that significantly differs from the group of intact animals and confirms the conservation of functional retinal activity in both cases.

Introduction of glibenclamide in animal groups with the correction of pathology led to decrease of the index b/a on 29 day of the experiment to a value, significantly different from the value of the group of intact animals, indicating on the blockade of the ATP-dependent potassium channels and confirms the preconditioning properties, in particular, minoxidil in a dose 0.5 mg/kg on the model of retinal angiopathy of hypertensive type.

Reducing of the ratio b/a in animals with simulated pathology caused by inhibition of the positive b-wave, which indicates violation of electrophysiological function of bipolar and Muller cells, as well as the possible contribution of the horizontal and amacrine cells. Saving of the electrophysiological function of the photoreceptor layer is confirmed by the absence of adverse changes of a-wave (fig. 6).

The observed changes in the functional activity of the retina during the ERG in modeling of retinal angiopathy of hypertensive type confirm the adequacy of the proposed model of pathology.

### Discussion.

The main factors in the development of retinal angiopathy of hypertensive type are disorders of common hemodynamics, local changes in the vessel walls. From local changes are the most important violations of the vascular endothelium [20].

In this regard, there is a need to find new methods of retinoprotection for possible reduction of the damaging effect of ischemia, formed in the retinal angiopathy of hypertensive type. Segment of drugs for the treatment of vascular diseases of the eye as a complication of systemic diseases is expedient to expand due to the increasing of incidence and lack of funds for targeted correction of ischemic lesions of the eye vessels [21].

Drugs used for correction of retinal ischemic damages are nonspecific therapy and do not have the desired result, that problem can be solved by pharmacological preconditioning, which is able to protect the retina from ischemic injury [22]. Versatility of preconditioning mechanism gives the background to the study of this phenomenon on the retina.

ATP-dependent potassium channel opening during ischemia plays a central role in the mechanism of cytoprotective effects of ischemic preconditioning. Initially, their activity was detected at sarcolemmal membrane, and later at the mitochondrial level.

Based on the fact that electrophysiological studies often have a decisive importance in the early and differential diagnosis of retinal disorders [23], to study the correction of functional changes in the retina, researcher must conduct a comprehensive analysis, including ophthalmoscopic, electroretinography, microcirculation research. Analysis of the dynamics of retinal electrogensis allows to evaluate the nature and topography of retinal disorders, as well as to identify the most labile hypoxic retinal structure, their reaction to the correction by the medications.
The foregoing predetermined the need to develop and systematize the methodological approaches to the assessment of the functional state of the retina and the subsequent optimization of correction of retinal angiopathy of hypertensive type. The studies conducted in our experiments on Wistar rats had developed a set of methodological approaches to assess the functional status of the retina, including instrumental methods of analysis (ophthalmoscopy, LDF, ERG).

The first step in exploring the possibility of correction of retinal angiopathy of hypertensive type using distant and pharmacological preconditioning by minoxidil, sildenafil has been the development of model of retinal angiopathy of hypertensive type on Wistar rats. We evaluated the fundus changes, the microcirculation level in the retina and its electrophysiological condition.

Simulation of retinal angiopathy of hypertensive type performed by administration of nonselective NO-synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) in a dose 12.5 mg/kg body weight of rat for 28 days.

28 days to Wistar rats corresponds to about 3.5 years for humans. During this time, the person on a background of hypertension has generated retinal vascular changes of hypertensive type. Hypertensive neuroretinopathy formed over decades, often developing in the late period of hypertensive disease and usually is a poor prognostic sign. It is characterized not only by changes in the blood vessels and retinal tissue, and involvement in the process of the optic nerve, which becomes swollen.
increased in size, swelling extends to the retina. Around the disk and on it hemorrhages are marked. Ophthalmoscopic picture is similar to the symptoms of stagnant disc, but unlike it marked a dramatic violation of color vision, decreased visual function: the decline of central vision and the narrowing of the field of view. At the end of neuroretinopathy the atrophy of the optic nerve may develop.

Summarizing the above, it should be noted that the proposed methodical complex of evaluation of functional changes associated with the development of retinal angiopathy of hypertensive type, makes possible to sufficiently evaluate objectively the retinoprotective effects of pharmacological agents.

The experiment showed that minoxidil in a dose 0.5 mg/kg prevented the development of ischemic damage and retinal vascular changes of hypertensive type caused by the introduction of L-NAME for 28 days, to a greater extent than sildenafil and distant ischemic preconditioning. It was revealed that in experimental group of animals treated with minoxidil in a dose 0.5 mg/kg was a significant difference of all measured parameters (the integral evaluation of the fundus changes, detected during ophthalmoscopy, the level of the microcirculation in the retina, the values of the coefficient b/a of electroretinogram) from value in the control group, which makes possible to talk about the ability of minoxidil in a dose 0.5 mg/kg to exert retinoprotective action on model of retinal angiopathy of hypertensive type in experiment.

The coefficient b/a of ERG in groups with correction by sildenafil and DIP statistically significantly different from the value in the group of intact animals, which makes impossible to speak about full retinoprotection, which is observed in the group of animals treated with minoxidil.

In proof of protection of retinal layers due to the effect of preconditioning of study drugs served the additional administration of glibenclamide in a dose 5 mg/kg, blocking ATP-dependent potassium channels, resulting in the elimination of the observed retinoprotective effects and confirms the preconditioning effects of minoxidil in a dose 0.5 mg/kg and sildenafil in a dose 0.5 mg/kg on the model of retinal angiopathy of hypertensive type.

Thus, the prospects become apparent to optimize pharmacotherapy of conditions accompanied by retinal ischemia, which are closely linked with the task of forming the methodology of the study antiischemic activity of pharmacologic agents based on an adequate assessment of the functional condition of the retina by instrumental methods of analysis.

Conclusion.

As a result, we developed a model of retinal angiopathy of hypertensive type. An integrated assessment of the fundus, the level of microcirculation, retinal electrophysiological state have been carried out, that allows us to appreciate fully the functional condition of the retina in the modeling of pathology and its correction.

This model of pathology allowed to estimate the possibility of preconditioning of retina by minoxidil in a dose 0.5 mg/kg, by sildenafil in a dose 0.5 mg/kg compared to the distant ischemic preconditioning. Intragastric administration of minoxidil ni a dose 0.5 mg/kg 60 minutes before the administration of L-NAME in odd days of experiment (every 48 hours) resulted in prevention of ischemic injury and retinal vascular changes of hypertensive type, significant increase in the level of microcirculation, preservation of electrophysiological retinal activity more than sildenafil in a dose 0.5 mg/kg and distant ischemic preconditioning.

Prior administration of glibenclamide in a dose 5 mg/kg eliminated the positive effects of the study drugs and DIP, which confirms the implementation of retinoprotection by preconditioning, carried out with the participation of ATP-dependent potassium channels.

References

8. Das B., Sarkar C. Is the sarcolemmal or mitochondrial K (ATP) channel activation important in the

9. Efremenkova D.A. Influence of distant ischemic and pharmacological preconditioning by nikorandil and by minoxidil on a skin patch survival on the pedicle and the state of the microvasculature (experimental research) [Text]: Author. Dis. ... cand. biol. sciences: 03.00.02 / TS


19. Konstantinova, TS. Tread and neurotoxic role of nitric oxide in the models of visual pathologies [Text]: Author. Dis. ... cand.biol. sciences: 03.00.02 / TS

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Yakusheva E.N.1, Engalycheva E.E.2, Sychev I.A.3, Shcul'kin A.V.4 PHARMACOLOGICAL EVALUATION POLYSACCHARIDE COMPLEX FLOWERS TANSY

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Abstract. From flowers tansy extracted polysaccharide complex. Installed its qualitative and quantitative composition, have developed a technique standardizing the content of reducing sugars. By thin layer chromatography and high pressure liquid chromatography after acid hydrolysis installed monosaccharide composition: glucose, xylose, arabinose, galactose and mannose. It is proved that the polysaccharide has a high content of uronic acid, which allows it to include the class of pectin. The investigation of gastroprotective activity of polysaccharide in the prophylactic administration at model destruction of the gastric mucosa to indomethacin. Introduction polysaccharide prevents various types of erosive and ulcerative destruction. According to anti-ulcer activity of the drug is superior to ranitidine and comparable to omeprazole. The study of anti-inflammatory activity of the polysaccharide on the model of exudative inflammation caused by the introduction of the formalin solution under aponeurosis posterior limbs rat. The polysaccharide after oral administration reduces the edema of inflamed tissues limbs, reducing leukocytosis, erythrocyte sedimentation rate normalizes. Anti-inflammatory activity comparable with diclofenac sodium. The study of hepatoprotective activity of the selected polysaccharide on the model of toxic carbon tetrachloride liver lesions. Polysaccharide complex reduces the level of aspartate aminotransferase and alanine aminotransferase; by hepatoprotective activity inferior silymarin. The study of antioxidant activity was conducted on the model of acute toxic hepatitis and exudative inflammation model. Polysaccharide complexes exhibit pronounced antioxidant effect, it reduces the concentration of TBA-reactive products, increases the content of protein-free thiol groups and glutathione peroxidase activity. When coadministered with diclofenac sodium eliminates its prooxidant action.

Keywords: polysaccharaide, flowers of Tansy, anti-inflammatory activity, antioxidant effect, gastroprotective activity, hepatoprotective activity.

Introduction. A promising direction in the developing of pharmacology is the research of new drug sources, including medicinal plants. Medicinal herbal preparations have a number of advantages by the condition of efficient use: they have diverse pharmacological activity and sufficiently large breadth of therapeutic action, but at the same time, they have milder effect in comparison with synthetic drugs, especially it is important for long-term use; they are relatively safe and practically do not cause allergic reactions [1].

At the present time the most promising herbal remedies are considered drugs of individual substances, which are belonged to various classes of chemical substances such as alkaloids (atropine sulfate, codeine phosphate, pilocarpine hydrochloride), glycosides (digoxin, strofiantin K), flavonoids (rutin, quercetin).

Polysaccharides are used in medical practice, mostly in the form of herbal medicines. However, at the present time it is carried out the active screening of non-starch polysaccharides of higher plants and algae in order to develop effective drugs on the basis of individual substances [2, 3].

Non-starch polysaccharides have diverse effect on the body normally and in various pathological conditions. They have adaptogenic effect [4], stimulate the physical performance [5], and the immune system [6, 7], enhance phagocytosis,
increase the production of antibodies, increase the number of lymphocytes in blood, demonstrate the anti-inflammatory activity [8, 9]. On the surface of the stomach mucosa and bowel polysaccharides form the gel which makes ambient and protective effect [10], moreover by means of swelling they modify the transit of chymus through the gastrointestinal tract, polysaccharides have a prebiotic activity [11], and they are promising enterosorbsents [12].

Despite the relatively large amount of information about the biological activity of plant polysaccharide complexes, practically there are no publications reflecting the interaction between the characteristics of the specific polymer’s structure, and the presence and the degree of their pharmacological action. In addition, one of the most important issue is the clarification and the elaboration of some possible mechanisms of polysaccharides action and their complexes in the treatment and prevention of various types of pathologies.

Tansy – is the officinal herb, which is used in medicine as a choleretic agent. In medical practice, they use the flower’s infusion, as well as dry extract - drug "Tanatsehol". Tansy flowers have a complex chemical composition. They contain flavonoids, essential oils, hydroxycinnamic acids, tannins, polysaccharides [13].

The aim of the research was to separate polysaccharide complex from flowers of tansy and to study its composition and pharmacological activity.

Materials and methods.

Polysaccharide complex flowers tansy (PSP) isolated from air-dried flowers tansy ("Flowers of Tansy", "Zdobor’e" Ltd., Russia). Polyphenolic compounds isolated by preliminary extraction with 40% ethanol. From the resulting seed meal extraction three times with 1% ammonium oxalate solution for 1.5 hours recovered PSP, which was precipitated with excess ethanol. Extraction purification was done by successive washing with ethanol, acetone and ether. This production method makes better use of medicinal herbs as a polysaccharide is used as a source of meal after separation of flavonoids. A method for producing patented in the Russian Federation. Polysaccharide complex authenticity confirmed by qualitative reactions, identified pH, kinetic viscosity of a 3% solution and its solubilizing ability [14].

PSP monosaccharide composition was determined after acid hydrolysis by thin layer chromatography and high effective liquid chromatography. Uronic acids content was determined gravimetrically by precipitation reaction of polygalacturonic acid in the form of calcium pectate. The content of free carboxyl groups was determined alkalimetrically (phenolphthalein indicator). A technique has been proposed to standardize the PSP, based on the spectrophotometric determination of the content of reducing sugars by reaction with picric acid after acid hydrolysis of the polysaccharide [15].

The study of the pharmacological activity of the PSP was carried out on rats 126 outbred line SD (Sprague Dawley), weighing 150 - 200 g, obtained from the nursery “Stolbovaya”, contained in the standard vivarium conditions. Animal studies performed in accordance with the "Rules of work with the use of experimental animals" (Order of the Ministry of Health 708n dated 23.08.2010).

Investigation of gastroprotective activity was conducted on 35 male rats, divided into 5 groups of 7 animals each. Ulcer simulated by intragastric administration of indomethacin (Indomethacin, "Sofarma" Ltd., Bulgaria) at a dose of 20 mg/kg twice at an interval of 4 hours [16]. The first group of animals administered daily PSPs for 3 days, twice a day for 4 – one hour before the 1st and 2nd indomethacin. Efficacy was evaluated 16 hours after the ulcerogenic action of the agent as a result of a macroscopic study on the presence of gastric ulcers. Destruction differentiated by size, counted Pauls index and anti-ulcer activity of the drug. The second and third group of animals was administered the reference drugs – omeprazole (Omeprazole, "Promed" Ltd., Russia) at a dose of 20 mg/kg [17] and ranitidine (Ranitidine, "Ozone" Ltd., Russia) at a dose of 25 mg/kg [18] mode purpose polysaccharide.

Study of anti-inflammatory activity was performed on 56 animals were divided into 4 groups of 14 animals each. 0.1 ml of 2.5% formalin solution was injected for the simulation of the inflammatory response by the fascia of the right hind limb rat [19]. The first group of animals was administered intragastrically PSP 2 hours before the injection of formalin solution, followed by 2 hours after injection, the next 7 days once. The second group of animals was administered reference drug diclofenac sodium (Diclofenac, "Hemofarm" Ltd., Russia) at a dose of 11 mg/kg [20] in the mode of appointment of the polysaccharide. A third group of animals in the same period was administered a combination of PSP at 0.3 g/kg body weight and diclofenac sodium in a dose of 11 mg/kg body weight. After 1, 2, 3, 4, 6 and 24 hours after the administration of formalin, then once a day for 7 days was conducted onkometric measurement values limb edema in rats on a digital plethysmometer StoeletingCo (USA). After 4 hours at 3 and 8 hours after the start of the experiment in all groups was determined leukocyte level and ESR value [21].
The study of hepatoprotective activity was conducted on 28 male rats divided into 4 groups of 7 animals each. Simulation of acute toxic hepatitis was performed administration the oil 50% carbon tetrachloride solution intragastrically at a dose of 0.1 ml per 100 g body weight twice a day – control pathology series \[19]. Beginning 1 day after the last administration of CCl₄ first group of animals on a daily basis for 7 days was administered PSP at 0.3 g/kg body weight. On the 8th day (24 hours after the final injection) evaluated the effectiveness of the drug on the following parameters: the activity of AST (U/l), ALT (U/l) and alkaline phosphatase (U/l), the level of total and direct bilirubin (mmol/l). Animals of the second group received a comparison drug – fruit extract of milk thistle ("Karsil", "Sopharma" Ltd., Bulgaria) - the equivalent of silymarin – 100 mg/kg in the polysaccharide assignment mode \[22].

Effect of polysaccharide complex tansy flowers on the state of lipid peroxidation (LPO) and antioxidant defense cells was evaluated in two models of acute liver toxicity and acute exudative inflammation at the level of TBA-reactive products (TBA-RP), thiol groups (of GSH), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) lysate of erythrocytes and blood plasma. The test parameters were determined as follows: RP-TBA concentrations (nmol/mg protein) by the reaction with thiobarbituric acid (method Stalnoy D.I., Gorishvili T.G. 1977), the level of GSH (mol/mg protein) by reduction reaction disulfide-5,5-dithiobis-2-nitrobenzoate (method Habeeb A.F.S.A., 1972) on spectrophotometer Shimadzu UV-150-02, GPx activity (nmol NADH/min × mg protein) by enzymatic reduction reaction of tert-butyl hydroperoxide glutathione (method Paglia D.E., Valentine W.N., 1967, modification Larkin V.Z.), GST activity (HDBN nmol/min × mg protein) by the reaction of conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (method Keen J.N., Iakoby W.B., 1978) using biochemical analyzer Humalyzer 2000.

Statistical processing of the results obtained in the course of chemical research carried out under the requirements of the State Pharmacopoeia of the Russian Federation XIII edition. Statistical processing of the results obtained in animal experiments were carried out using applications MS Exel 2010 and Statsoft Statistica 8.0. The data are shown as the arithmetic mean and standard error of the average value of (M±m) data in the normal distribution and a median upper and lower quartiles - in the distribution data other than the normal. The nature of the data distribution was assessed by the Shapiro-Wilk. The presence of a statistically significant between-group differences were determined using one-way ANOVA, differences between groups were determined by the criterion of Newman-Casely, reliable results are considered at a significance level of p <0.05 \[23].

**Results.**

Polysaccharide complex extracted from tansy flowers, amorphous material is light gray, in which the total content of the feed is 6.5%. The viscosity of the solution as measured under standard conditions was 2,2 Pa·sec, pH=6,70. PSP has a solubilizing ability for hydrophobic dyes. The polysaccharide is substantially free of free mono- and disaccharides in their determination by high performance liquid chromatography. According to the results of thin layer chromatography and high performance liquid chromatography, it was determined that the composition comprises PSP glucose, galactose, xylose, arabinose and mannose. Uronic acids content of 84,7±1,28%, free carboxyl groups – 15,66±4,79%; standardization of PSP held on the content of reducing sugars.

Introduction of indomethacin at a dose of 20 mg/kg twice at intervals of 4 h resulted in the formation of erosive-ulcerous lesions in 100% of the animals of all groups (table 1). Grossly destruction in rats differentiated into large (7,43±4,79), strip (5,00 ± 3,27) and chiseled (10,71 ± 2,93), the index of Pauls (for its calculation of the average number of ulcers was multiplied by the percentage of animals with ulcers, and divided by 100%) was 23,14.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of Ulcers in Animals Treated with a Dose of 20 mg/kg for 4 Days</th>
<th>Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSP</td>
<td>248</td>
<td>p&lt;0,05</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>245</td>
<td>p&lt;0,05</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>244</td>
<td>p&lt;0,05</td>
</tr>
</tbody>
</table>

Number of destructions in animals treated with a dose of PSP 0,3g/kg for 4 days course, reliable decreased compared to control data: the number of large ulcers decreased to 2,48 times, chiseled – in 1.6 times, strip-ulcer attended only one animal.

Antiucler activity (ratio Pauls index in the control group to Pauls index in the experimental group), PSP was 2,31, indicating a marked gastroprotective effect at prophylactic use.

Introduction comparator drugs ranitidine and omeprazole reduced the number of large ulcerations in 3,72 times (p<0,05) and 6,52 times (p<0,05), chiseled destructions of 1,32 times and 1,41 times (p<0,05) respectively. In both groups significantly decreased the number of strip ulcers are observed upon administration of ranitidine in two animals, when administered omeprazole – one. Antiulcer activity of ranitidine was 2,25; omeprazole – 2,49.
**Table 1.**

<table>
<thead>
<tr>
<th>Series animals</th>
<th>Number of destructions 1 animal</th>
<th>Index Pauls</th>
<th>Antiulcer activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large</td>
<td>Strip</td>
<td>Chiseled</td>
</tr>
<tr>
<td>Control pathology, n=7</td>
<td>7,43±4,79</td>
<td>5,00±3,27</td>
<td>10,71±2,93</td>
</tr>
<tr>
<td>PSP, n=7</td>
<td>3,00±1,63*</td>
<td>0,00</td>
<td>6,71±1,49*</td>
</tr>
<tr>
<td>Ranitidine, n=7</td>
<td>2,00 (0,00;2,00)*</td>
<td>0,00 (0,00;3,00)*</td>
<td>8,14±2,91</td>
</tr>
<tr>
<td>Omeprazole, n=7</td>
<td>1,14±1,21*</td>
<td>0,00</td>
<td>7,57±1,99*</td>
</tr>
</tbody>
</table>

Note: * - p<0,05; ** - p<0,005 - comparison of data from animal disease group control pathology

The introduction of formalin induced inflammatory response, accompanied by hyperemia and edema of the limbs in animals of all groups. The maximum value of edema in rats of the control group was observed after 4 hours after injection of formalin. In subsequent periods of observation, the gradual reduction of edema, limb volume normalization was observed on the 7th day of the experiment (figure 1).

The development of an inflammatory response accompanied by severe leukocytosis. After 4 hours after the administration of formalin, the number of leukocytes in rats of the control group increased by 3 times compared to the intact animals (p<0,05). ESR level in this group peaked on the third day of research and was 133,3% (p<0,05) relative to the level of intact animals.

The dynamics of edema in animals treated with PSP corresponded to that in the control series pathology, but its intensity decreased significantly. After 4 hours after the administration of formalin edema value significantly decreased by 8,97%, the level of leukocytes and ESR decreased by 38,67% (p<0,05) and 5,88% respectively, compared with the control group data. Normalization of these indices took place on the 7th day of research.

![Figure 1. Dynamics of limb edema development (as a percentage of the norm), caused by the introduction of the formalin, without treatment and with therapy. * - p<0,05 – comparison of data from animal disease control group.](image)

Appointment of diclofenac sodium contributed significantly delay the development of the inflammatory process. As compared to the control group of rats, the intensity of edema disease through 4 hours after administration of formalin authentically decreased by 7,24%, the level of white blood cells to 52,79% (p <0,05) and ESR value is not changed.
Normalization parameters observed on the 5th day of the experiment.

The introduction of a combination of PSP and diclofenac sodium were not significantly changed the dynamics of limb edema as compared with the animals treated with diclofenac sodium and cap separately. The level of leukocyte and erythrocyte sedimentation rate was comparable with figures of animals treated with diclofenac sodium.

In intact animals the activity of AST was 81,03±6,02 U/l, ALT – 23,00±2,00 U/l, alkaline phosphatase – 306,33±14,74 U/l, the content of total bilirubin 0,1 (0,1; 0,11) mmol/l, direct bilirubin 0,033 (0;0,100) mmol/l.

The double introduction of carbon tetrachloride in rats led to development of acute toxic hepatitis. The animals of the control group in the plasma enzyme activity was statistically increased as compared to the intact animals: AST – 1,52 times, ALT – in 2,48 times, alkaline phosphatase (AP) – 2,73 times (figure 2) has also increased the content of total bilirubin 2-fold (p<0,05) and direct bilirubin in 2,51 times.

Figure 2. Activity (percentage of normal) of liver enzymes in the blood plasma at the 8th day in rats with hepatitis and during treatment with PSP and silymarin.

Hereinafter: * - p <0,05 – animals compared with data intact animals; ** - p <0,05 – comparisons with the data of disease control.

Introduction PSP at 0,3 g/kg for 7 days, reduced the toxic liver damage caused by carbon tetrachloride. ALT and AST activity was reduced compared to control in the pathology 1,31-fold (p<0,05) and 12,67% respectively.

Course administering silymarin 100 mg/kg also resulted in reduced CCl₄ toxicity in the liver. The animals of this group on the 8th day study statistically significantly decreased content of AST, ALT and total bilirubin (2,56; 1,48 and 2 times, respectively) in the blood plasma as compared to control these diseases.

Changes in the antioxidant defense system cells under the influence of PSP has been evaluated in two models: acute toxic hepatitis and acute exudative inflammation by Selye.

Indicators of the state of lipid peroxidation and antioxidant protection in intact animals are presented in table 2.

Toxic hepatitis, caused by the introduction of a 50% oil solution of carbon tetrachloride, was accompanied by the activation of the LPO. In the control group of rats TBA-RP blood plasma on the 8th day after the last injection of CCl₄ was statistically increased in 1,56 times, GSH levels in the lysate of erythrocytes and blood plasma decreased to 2,08 (p<0,05) and 1,56 (p<0,05) times, respectively compared with those of intact animals. Simultaneously there was a statistically significant decrease in the activity of GPx and GST in erythrocyte hemolysate 2,40 and 2,44 times, respectively (table 2).
**Table 2.**

<table>
<thead>
<tr>
<th>Series animals</th>
<th>Intact animals, n=7</th>
<th>Control hepatitis, n=7</th>
<th>PSP course of 7 days, n=7</th>
<th>Silymarin course of 7 days, n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA-RP hemolysate</td>
<td>6.90±0.55</td>
<td>13.89±5.50</td>
<td>9.01±1.06*</td>
<td>7.48±1.94</td>
</tr>
<tr>
<td>TBA-RP plasma</td>
<td>29.12±1.97</td>
<td>45.41±8.57*</td>
<td>31.70</td>
<td>33.84</td>
</tr>
<tr>
<td>GSH hemolysate</td>
<td>128.70 (128.22;129.18)</td>
<td>61.86±20.68*</td>
<td>135.5±24.76**</td>
<td>79.94±29.05</td>
</tr>
<tr>
<td>GSH plasma</td>
<td>102.49±13.37</td>
<td>65.50±9.79*</td>
<td>73.52±11.01*</td>
<td>85.78±10.68**</td>
</tr>
<tr>
<td>GST hemolysate</td>
<td>12.85±4.53</td>
<td>5.36±0.74*</td>
<td>7.67±2.12**</td>
<td>5.11 (4.46;23.24)*</td>
</tr>
<tr>
<td>GST plasma</td>
<td>257.85 (238.80;276.90)</td>
<td>210.95±41.30</td>
<td>176.83±31.10*</td>
<td>219.63±67.43</td>
</tr>
<tr>
<td>GPx hemolysate</td>
<td>1.22±0.41</td>
<td>0.50±0.17*</td>
<td>0.91±0.34</td>
<td>0.93±0.29</td>
</tr>
<tr>
<td>GPx plasma</td>
<td>36.05 (31.50;40.60)</td>
<td>22.18±15.43</td>
<td>19.00±4.57*</td>
<td>27.07±2.11</td>
</tr>
</tbody>
</table>

The intensity of lipid peroxidation in animals treated with PSP at 0.3 g/kg of body weight 7 days course was less pronounced as compared to control disease. The level of TBA-RP hemolysate of red blood cells was reduced by 35.13% and 119.12% (p<0.05) increased the content of protein-free thiol groups, increased the activity of GST and GPh at 32.14%, the content of GSH increased by 47.29% (p<0.05). The rest of indicators studied were not statistically different from the intact animal data suggesting that the decrease in the processes of lipid peroxidation and antioxidant defense stimulation PSP.

As compared to intact animals treated rats at a dose of PSP 0.3 g/kg of body weight 7 days of course, on the 8th day of TBA-RP erythrocyte hemolysate level remained statistically authentically increased to 30.58%, the content of GSH in blood plasma was lower by 28.27% (p<0.05) and GPx activity – by 47.29% (p<0.05). The rest of indicators studied were not statistically different from the intact animal data suggesting that the decrease in the processes of lipid peroxidation and antioxidant defense stimulation PSP.

Course introduction silymarin also led to a decrease in the severity of lipid peroxidation. The level of TBA-RP in hemolysate of red blood cells in rats in this series decreased by 46.14% (p<0.05), plasma – at 25.47% (p<0.05). Statistically significant increased content of protein-free thiol groups in blood plasma at 30.96%, GST activity was decreased by 4,66% in erythrocyte hemolysate and increased by 4.11% in the blood plasma as compared control diseases, GPx activity in erythrocyte hemolysate and in the blood plasma was increased by 86.00% and 22.05% respectively. Compared to intact animals when administered silymarin GST activity remained reduced to 60.23% (p<0.05), other indicators were not statistically different from the normal level.

Introduction formalin under foot aponeurosis of rats led to an increase in lipid peroxidation processes and a reduction in the antioxidant defense of cells. The animals in the control group 4 hours after formalin injection of TBA-RP levels in hemolysate of red blood cells increased by 35.29% (p<0.05), GST activity increased by 18.05%, the content of thiol groups is decreased by 19.41% (p<0.05) GPh activity – by 50.69% compared with those of intact animals (table 3). The blood plasma statistically significantly increased TBA-RP content to 71.28%, other indicators have changed in different directions and not statistically significant.

Introduction PSP at a dose of 0.3 g/kg of course 7 days reduced the intensity of lipid peroxidation and increased antioxidant protection of cells: compared with the control of disease in 4 hours after the injection of formalin level of TBA-RP in hemolysate of red blood cells in rats decreased by 40.43% (p<0.05), the content of protein-free thiol groups authentically increased by 202.57%, the GST activity increased by 7.75% and GPh to 127.97% (p<0.05). The blood plasma is a statistically significant decrease in the concentration of TBA-RP at 32.14%, other indicators have changed not statistically significant. When comparing these results with the data of intact animals revealed normalization on the background of PSP levels of TBA-RP and protein-free thiol groups, the activity of GST and GPh in hemolysate of red blood cells increased by 10.73% and 40.08%, respectively.
Effect of polysaccharide complex flowers of tansy, diclofenac sodium, and combinations thereof on the state of lipid peroxidation and antioxidant protection in rats with experimental exudative inflammation.

Table 3.

<table>
<thead>
<tr>
<th>Series under investigation indicator</th>
<th>Intact animals, n=7</th>
<th>Control inflammation after 4 hours after administration of formalin, n=7</th>
<th>PSP, 4 hours after the administration of formalin, n=7</th>
<th>Diclofenac-sodium, 4 hours after the administration of formalin, n=7</th>
<th>The combination of PSP and diclofenac sodium in 4 hours after the administration of formalin, n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA-RP hemo-lysate plasma</td>
<td>8,49±1,06</td>
<td>11,51±1,69*</td>
<td>9,13±1,24**</td>
<td>10,4±0,54</td>
<td>11,2±0,59</td>
</tr>
<tr>
<td>GSH hemo-lysate plasma</td>
<td>17,13±9,22</td>
<td>29,34±3,49*</td>
<td>19,91±1,57**</td>
<td>7,71±4,06**</td>
<td>7,07±3,88**</td>
</tr>
<tr>
<td>GST hemo-lysate plasma</td>
<td>154,14±44,81</td>
<td>60,29±48,17*</td>
<td>182,45±22,44**</td>
<td>93,11±28,89</td>
<td>115,85±18,91</td>
</tr>
<tr>
<td>GPx hemo-lysate plasma</td>
<td>106,04±27,92</td>
<td>129,93±30,22</td>
<td>91,64±13,04</td>
<td>74,41±30,96</td>
<td>78,42±13,10**</td>
</tr>
<tr>
<td></td>
<td>12,46±1,87</td>
<td>14,71±2,26</td>
<td>15,85±2,77**</td>
<td>12,19±3,18</td>
<td>13,98±3,24</td>
</tr>
<tr>
<td></td>
<td>188,75±29,98</td>
<td>181,40±33,00</td>
<td>209,00±27,65</td>
<td>254,67±23,73***</td>
<td>217,40±40,21</td>
</tr>
<tr>
<td></td>
<td>2,37±0,47</td>
<td>1,43±0,18</td>
<td>3,32±0,43**</td>
<td>2,23±0,42**</td>
<td>2,04±0,24**</td>
</tr>
<tr>
<td></td>
<td>41,42±7,01</td>
<td>34,75±7,87</td>
<td>44,05±18,03</td>
<td>45,20±3,82**</td>
<td>45,23±5,41**</td>
</tr>
</tbody>
</table>

Course introduction of diclofenac sodium in a dose of 11 mg/kg for 7 days increased the intensity of lipid peroxidation. After 4 hours after the initiation of the inflammatory reaction against treatment diclofenac sodium TBA-RP levels remained elevated erythrocyte hemolysate (disease level control), the content of thiol groups increased by 39,97%, GST activity has decreased by 17,13% (p<0,05), GPh - increased by 56,64% (p <0,05) relative to control edema. Comparison of the results of this series with the data intact rats showed that TBA-RP levels remained elevated at 22,35%, a protein-free thiol groups was reduced by 45,23%, the GST activity and GPh remained lowered. Thus, diclofenac sodium has prooxidant effect early in the inflammatory response.

Co-administration of PSP and diclofenac sodium 4 hours after formalin injection resulted in a significant increase in the content of erythrocyte hemolysate thiol groups at 92,15%, TBA-RP level has not changed, GPh activity increased by 36,00% (p<0,05), GST – has not changed relative to control edema. The plasma TBA-RP levels decreased by 75,90% (p<0,05), the content of thiol groups to 39,64% (p<0,05), glutathione peroxidase activity increased by 30,16% (p<0,05), the activity of glutathione-S-transferase is not significantly increased. As compared to the intact animals showed a significant increase of TBA-RP levels in hemolysate at 31,92%, reduction of protein-free thiol groups on 24,84%, GST activity increased and decreased GPh.

On day 7, the study of the control group animals TBA-RP levels in hemolysate of red blood cells remained significantly lower at 29,96% (p<0,05), in plasma, this figure does not change significantly. In all treatment groups studied parameters on the 7th day were close to the data of intact animals and statistically not different from them.

Discussion.

Dedicated extraction with a 1% solution of ammonium oxalate, polysaccharide complex flowers tansy is an amorphous light gray. When dissolved in water, it forms a viscous solution having a neutral pH. By thin layer chromatography and high performance liquid chromatography revealed that the composition comprises a polysaccharide: glucose, xylose, arabinose, galactose and mannose. The high content of uronic acids (84,7±1,28%) can be attributed to a class of PSP is pectin. Thus 15,66±4,79% of monosaccharide residues contain a free carboxyl group, which may account for some aspects of the pharmacological activity of the polysaccharide. Standardisation of the selected polysaccharide complex tansy flowers were carried out on the content of the amount of reducing sugars after acid hydrolysis.

On models indomethacin ulceration was found that the polysaccharide complex tansy flowers in prophylactic gastroprotective reception has a marked effect reducing the amount of degradation. For comparison, we choose the most effective and widely used antisecretory drugs with different mechanisms of action – omeprazole and ranitidine [24]. Comparative evaluation of the effectiveness of anti-ulcer activity coefficient showed that PSP is superior to ranitidine, but inferior to omeprazole.
3% solution of tansy flowers polysaccharide complex used in the experiments has a high kinematic viscosity and when administered in stomach envelops its walls, preventing the destructive action of hydrochloric acid on the mucosa. Pectic polysaccharides are surfactants [25] and can be oriented certain way of an interfacial of the hydrophilic and hydrophobic environment of the membrane of the stomach mucosa cells. The polysaccharide forms a protective film on the surface, which prevents corrosive environmental factors.

PSP, like other pectins, is a weak electrolyte, wherein it contains 15,66±4,79% of free carboxyl groups. Polysaccharide dissociation in the stomach is suppressed, molecular shape is formed, the concentration of hydrochloric acid is reduced. Thus, the possible effect of the antacid is another component providing gastroprotective action.

Plant polysaccharides exert wound-healing effect and stimulate tissue regeneration [26]. Calcium pectate in the experiment increases levels of proteolytic enzymes and concentration of nucleic acids [16], which according to the authors leads to activation of the synthesis of additional cells of the stomach on the principle of feedback. In view of the similarity of the structure can assume the existence of such action in the pectin polysaccharide flowers of tansy. In addition, PSP has an antioxidant effect, which reduces mucosal damage by free radicals formed as a result of increased lipid peroxidation under the action of indomethacin.

Study of anti-inflammatory activity of complex polysaccharide tansy flowers were carried out on the model of exudative inflammation caused by the introduction of a solution of formalin under the aponeurosis of the hind limbs of animals. The introduction of PSP in a dose of 0.3 g/kg rate to 7 days resulted in a marked inhibition of edema and exudation reduction, reduced leukocytosis and ESR normalized. As a comparison, the drug used most widely used NSAIDs – diclofenac sodium. Anti-inflammatory activity of the polysaccharide complex tansy flowers in the experiment was comparable to the effect of diclofenac sodium. However, he, like other NSAIDs, as a pro-oxidant has a damaging effect on the gastric mucosa. Therefore it was of interest to explore the possibility of co-administration of drugs, to identify a possible increase anti-inflammatory activity and to prevent adverse effects of diclofenac sodium.

Simultaneous administration of PSP and diclofenac sodium does not significantly alter the dynamics of the limb edema compared with separate administration. The level of leukocyte and ESR were not significantly different from that of animals treated with the polysaccharide and NSAID separately. Thus, we can conclude that there is no potentiation of the anti-inflammatory effect when administered to a combination of drugs. However, the use of such combinations is partially eliminates the prooxidant action of diclofenac sodium in the early period of development of the inflammatory response, as evidenced by the increase in free sulfhydryl groups and glutathione peroxidase activity in hemolysate of red blood cells.

The mechanism of antiinflammatory action PSP, as well as other pectin is probably related to activation of monocyte-macrophage system producing proteoglycans, glycoproteins and glucosamine, which leads to an acceleration of the maturation of T-and B-lymphocyte precursor cells. In turn, produces lymphocytes increases the activity of phagocytosis process. Under the influence of plant polysaccharide is an increase of plasmatic cells spleen and increased synthesis of γ-globulin, which also leads to the activation of phagocytosis. Polysaccharides are surfactants, they may interact with cell membranes, increasing the peroxide resistance [27]. In addition, PSP displays antioxidant activity, allowing you to reduce the damaging effects of free radicals, resulting in inflammation. This is consistent with the findings of our study: reduction of TBA-RP levels, increasing the number of free sulfhydryl groups and the activity of glutathione-S-transferase and glutathione peroxidase using PSP for the treatment of exudative inflammation. Integrated action reduces edema, normalization of leukocytes and ESR.

The study of hepatoprotective activity of complex polysaccharide tansy flowers were carried out on the model of acute toxic liver injury by carbon tetrachloride. Against the background of a course of PSP occurred statistically significant reduction in elevated levels of AST, however, ALT level decreased significantly not. Lowering transaminase activity indicates a decrease and stabilization of hepatocyte cytolysis membranes.

Silammarin was selected as comparison drug, because it is a widely used herbal hepatoprotectors. Underlying mechanism of action of silammarin is the stabilization of hepatocyte membranes, inhibition of cAMP, which leads to inhibition of calcium-dependent phospholipase and improving metabolic processes in the liver, increase in protein synthesis and cell regeneration acceleration. In addition, silammarin has antioxidant activity [28].

The effect on cytolysis polysaccharide complex flowers of tansy inferior silammarin. However, the
main role in protecting liver cells administered at course SAPs apparently plays its pronounced antioxidant activity.

As is known, carbon tetrachloride toxicity generally associated with the formation of free radicals during its metabolism in the cytochrome P-450 monooxygenase system. The indicators of free radical processes are intermediate oxidation products – diene conjugates and TBA-RP, and the concentration of the latter is directly proportional to the intensity of lipid peroxidation [28].

Introduction of the complex polysaccharide tansy flowers at 0.3 g / kg decreased the levels TBA-RP in hemolysate of erythrocytes and blood plasma, significantly increased the content of free thiol groups, and activity of glutathione-S-transferase in erythrocyte hemolysate. Free sulfhydryl groups play an important role in the redox homeostasis in the cell due to the ability to reversibly move from the reduced form (GSH) to oxidized (GSSG), changing the conformation, catalytic and regulatory functions of proteins [29].

Thus, the polysaccharide complex flowers of tansy has a strong antioxidant effect exceeding the activity of silymarin. PSP reduces the activity of lipid peroxidation and stimulates antioxidant cell system.

PSP, having a high potential for adsorption may bind free radicals and lipid peroxidation products formed when damaged cells, thereby providing a direct antioxidant effect and reducing the toxic load on the liver. By increasing the content of sulfhydryl groups and the activity of glutathione-S-transferase increases neutralizes function of hepatocytes, thereby realized indirect antioxidant effects.

Several studies have proved the presence of a stabilizing membrane activity of pectin polysaccharides [27]. The physiological effects on the cell membrane is mainly determined by the structure and physicochemical properties of the substance, as well as membrane receptors. The antioxidant effect can be realized by the presence of anti-inflammatory and immunomodulatory effect.

Conclusions.

1. The polysaccharide complex tansy flowers isolated on the patented technique, belongs to a class of pectin. According to the results of thin layer chromatography and high performance liquid chromatography it revealed that the polysaccharide composition comprises glucose, galactose, xylose, arabinose and mannose. Pectin fraction contains up to 85% polygalacturonic acid, 16% of which have a free carboxyl group. The method of standardization polysaccharide complex content of reducing sugars. Polysaccharide complex tansy flowers has a high solubilizing capacity. Its solution has high kinetic viscosity and neutral pH.

2. The polysaccharide complex tansy flowers at preventive oral administration for 4 days at a dose of 0.3 g/kg body weight has a significant gastroprotective effect of reducing the amount of destructive erosive and ulcerative lesions caused by indomethacin. In the experiment, antiulcer activity polysaccharide comparable to that of omeprazole and superior to that of ranitidine.

3. The polysaccharide complex tansy flowers when administered orally 7 days a course dose 0.3 g/kg has a pronounced inhibitory effect on the development of exudative component of the inflammatory response induced by administration of formalin aponeurosis limb rats, reduces leukocytosis and the level ESR. Polysaccharide complex inflammatory activity comparable with the activity of diclofenac sodium. Simultaneous administration of diclofenac sodium and polysaccharide complex flowers tansy does not significantly alter the dynamics of the limbs edema, leukocyte count and ESR value.

4. The polysaccharide complex flowers tansy has hepatoprotective effect, reduces cytolysis, reduces the activity of AST in the blood. The effect is inferior silymarin.

5. The polysaccharide complex flowers tansy exhibits anti-oxidant action, causing a reduction in the concentration of TBA-reactive products, elevated levels of protein-free thiol groups and the activity of glutathione-S-transferase and glutathione peroxidase in oxidative stress, caused by the introduction of carbon tetrachloride and inflammatory response. Polysaccharide complex by superior antioxidant activity of silymarin and reduces prooxidant effect of diclofenac sodium in a joint application early in the inflammatory response.

References


research. №10 (2014): 93-100. (In Russian) [eLIBRARY] [Fulltext]


21. Dolgov V.V., Men'shikov V.V. Clinical Laboratory Services. National leadership. T.1 (Moscow: GEOTAR-Media, 2012), 928 p. [eLIBRARY] [Fulltext] (In Russian)


25. Kaisheva N.S., Kaishev A.S. Surface-active properties of distillers grains and the pectins isolated from them. Pharmacy and Pharmacology. №1 (14) (2016): 72-84. [eLIBRARY] [Fulltext] (In Russian)


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PHARMACOTHERAPY EXACERBATIONS OF CHRONIC INFLAMMATORY CONDITIONS OF FEMALE GENITAL SPHERE USING TO GEPTON AND LONGIDAZA

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Abstract. Researches of the last years showed that changes of local immunity at inflammatory diseases of appendages at women, to be exact a condition of a secondary immunodeficiency at the local level, arising against the main pathological process, are a consequence of an immune inflammation, violation of structure and function of cellular membranes because of lipid peroxidation. Research objective was establishment of changes of the metabolic status at an aggravation chronic salpingoophoritis and detection of efficiency of use in «Gepon's» complex pharmacotherapy and «Longidaza's» various medicinal forms. For this purpose under continuous supervision was the 70th women aged from 20 till 35 years with the established diagnosis chronic salpingoophoritis in an aggravation stage. All patients were divided into four groups according to age, minimum satellite, severity of illness and providing treatment. The first experimental group was included 18 patients with chronic adnexitis in the exacerbation phase, where the standard therapy. The second group was included patients (17 women), which put on «Longidaza» in the form of suppositories (1 suppository 3000 per recti 1 times a day for 5 days). In the third group (18 patients), patients received, in addition to a standard treatment regimen «Gepon» (10 mg per 1 times a day for 5 days). The fourth group was included patients (17 women), which put on «Longidaza» injections (1 suppository 3000 intramuscularly 1 time per day for 5 days). As a result of a research efficiency of use of immunocorrective preparations («Gepon» and «Longidaza») at patients chronic salpingoophority in an aggravation stage in correction of metabolic frustration is established. «Longidaza's» use in the form of candles has corrective and normalizing impact on the broken indicators of the metabolic status at patients chronic salpingoophoritis at local level whereas purpose of an injection form of this preparation normalizes parameters of the oxidatic status at system level. Results of a research allow to draw a conclusion that application in complex pharmacotherapy of patients with chronic salpingoophoritis longidaza and gepon in comparison with standard treatment makes more expressed impact on clinical picture of disease and laboratory indicators at system and local levels, thus maximum efficiency possesses a preparation of longidaza in the form of injections.

Keywords: chronic salpingoophoritis, immune status, metabolic disturbances, gepon, longidaza

Introduction. In modern tearms of chronical inflammatory conditions, of the uterine appendages, are the actual problem of obstetrics and gynecology due to unexpressed clinical aspects. Extent of exposure, frequent relapses, leading to the development of adhesive processes, in the small pelvis, a breach of immunological reactivity and other changes [1, 2, 3, 4]. The systemic protection mechanisms, are diseases in cases of long-stretching and recurrent diseases of the uterine appendages against which it is most likely a violation of the pelvic organs with the development of the power of local tissue hypoxia [5, 6, 7]. There is a study about production, of inflammatory mediators of different nature a relatively new subject of study pathogenetic mechanisms of inflammation in the uterine appendages of women antioxidant protection, from which expression depends on changes in the level body, systems and organism as a whole [8, 9]. Recent researches have shown, changes in local immunity the state of the secondary immunodeficiency at the local level that occurs on a
background of the primary pathological process, are the result of immune inflammation, disruption of the structure and function of cell membranes due to lipid peroxidation [10].

The use of anti-inflammatory medication therapy has an active effect on the bacterial flora, but not enough to impair immune and metabolic status, which is opened the prospect of doing research to find effective ways and means of pharmacological immunorehabilitation [11].

Accordingly, only the use of standard therapy, doesn’t always lead to full recovery. So for the successful treatment of patients, with chronic inflammatory diseases, it must be integrated etiopathogenetic treatment, including an appropriate immune rehabilitation and prevention of adhesions [12].

As the results of previous researches using only immunomodulatory drugs in the correction of immune and oxidant disturbances that occur during exacerbation of chronic adnexitis is not enough, so in this respect it could be effective to use the drug with immunomodulating and enzymatic activity. Longidaza, being a derivative polyoxidonium in conjunction with hyaluronidase, and in a variety of pharmaceutical forms of its release [13, 14]. There are enough facts for appropriate effectiveness of the injection Longidaza, while suppository form of the drug has been understudied [15]. Also, it may be a promising application Gepon drug interferon inducer link immunity [16, 17].

On this basis, the development of pathogenetically substantiated pharmacotherapeutic strategy exacerbation of chronic adnexitis is one of the urgent problems of modern medicine.

Our objective: To identify the clinical and immunological effectiveness using complex processes, in the pharmacotherapy of acute exacerbations of chronic adnexitis Gepon and different dosage forms Longidaza.

Research objectives:

– To determine effects of Gepon on the immune status and lipid peroxidation in patients with chronic adnexitis in the exacerbation phase.

– To determine the effect of various dosage forms of pharmacotherapy, drug «Longidaza» on the immune status, and processes peroxidation, lipid in patients with chronic adnexitis in the exacerbation phase.

– To compare immunomodulatory and antioxidant effects «Gepon» and «Lonegidaza» in the form of suppositories or injectable solution in complex patients with acute exacerbation of chronic adnexitis.

– To compare the clinical and immunological effectiveness of methods, pharmacorrection including immunotrophic drugs in patients suffering from chronic adnexitis in the exacerbation phase.

– To identify correlations between clinical symptoms, and laboratory parameters for inclusion in a comprehensive standard pharmacotherapy, Gepon and Longidaza and an exacerbation of chronic adnexitis.

It was established clinical and immunological efficacy immunocorrective drugs («Gepon» and various forms of «Lonegidaza») in patients, with chronic adnexitis in the exacerbation phase. Using «Longidaza» in the form of suppository has a corrective and normalizing effect on the impaired performance immune status of patients with chronic adnexitis mostly at the local level, while the appointment of the injectable form of the drug normalizes immune parameters and oxidative status at the system level. The use of complex pharmacotherapy of patients with chronic adnexitis «Longidaza» and «Gepon» is compared with standard treatment and had more pronounced effect on the clinical symptoms disease and laboratory parameters on the system and local levels, with the maximum efficiency has drug injection «Longidaza».

There were effective complex methods for patients with chronic pharmacotherapy adnexitis in the exacerbation phase using «Gepon» or «Longidaza». Clinical and immunological efficacy of the applied sketch of pharmacotherapy of patients with chronic adnexitis is ascending in the following order: standard pharmacotherapy → standard therapy + «Gepon» standard therapy + «Longidaza» in the form of suppositories → standard therapy treatment + «Longidaza» in the form of injections.

There were significant interactions between indicators immunometabolic status on the system and local levels, and clinical symptoms that gives an indication of the effectiveness of the treatment, the dynamics of symptom exacerbation of chronic adnexitis and predicting disease outcome.

In clinical research it was used a team approach to the study of immunocorrective and antioxidant effects of immunomodulatory drugs in women of gynecological separation OBUZ «Regional Perinatal Center» in Kursk with chronic adnexitis in the exacerbation phase, in accordance with the recommendations of the World Health Organizations.

The learning of pharmacological corrective effects «Gepon» (LLC «Immapharma», Russia) and «Longidaza» («research and manufacturing association Petrovax Farm», Russia) on the immune, oxidative disorders and clinical symptoms in patients
with chronic adnexitis in the exacerbation phase was carried out using an enzyme multiplied immunoassay, laboratory methods for assessing oxidative indicators, activity of antioxidant systems and using widely certified methods of statistical data processing.

OWN RESEARCHES

Clinical impressions. There were 70 patients with chronic adnexitis in the exacerbation phase at the age of 20-35 years under constant surveillance on the basis of OBUZ «Regional Perinatal Center». Clinical exclusion was established on the basis of complaints, medical history, laboratory and physical examination [18, 19]. The control group consisted of 22 healthy volunteer donors of the same age. The inclusion criteria were:
– The age of 20-35 years;
– Medical history to 3 years;
– Certain disease is chronic adnexitis in the exacerbation phase;
– The severity of the state no more than moderate severity;
– Negative findings of STD;
– Tolerance of used drug ;
– A written consent to participate in doing research.

Exclusion criteria:
– Patients in serious and critical condition;
– Verified persons with specific STD;
– Persons with concomitant somatic pathology in the stage of incomplete remission and the exacerbation phase;
– Persons with an allergic reaction to the treatment;
– Patients who refuse to doing research.

In all cases, it was carried out direct microscopic for the exclusion of gonococcus infection (swabs were prepared then they were stained with 1% aqueous methylene blue and Gram) and biological (inoculation of medium for detecting Neisseria gonorrhoea) research of vaginal-cervical lavage.

To detect trichomonas it was carried out native and painted watercolor solution of methylene blue drugs, inoculation of medium for the culture diagnosis of trichomoniasis.

Diagnosis of chlamydia, mycoplasma, ureaplasma, gardnerela infection was producing by examining scraps obtained PCR using commercial kits firm «DNA diagnosis» All patients were subjected to testing for lues and HIV, which staged the standard serological tests. All patients made direct microscopic and bacteriological content of the Exploration of the cervical canal and urethra, performed a colposcopy, ultrasound investigation.

Laboratory methods of blood tests were carried out by conventional methods. It were taken hemogram as a basis for the physiological norm, corresponding to the international system of units (SI) in clinical trials.

All patients were divided into four groups according to age, minimum satellite, severity of illness and providing treatment (Table 1).

The first experimental group was included 18 patients with chronic adnexitis in the exacerbation phase, where the standard therapy is held: cefazolin (1.0 intramuscularly 4 times a day number 20), gentamicin (80 mg intramuscularly 3 times a day number 21), nystatin (500 thousand units inside. 4 times a day № 28) trichopolum (0,5 orally 3 times daily № 15) indometacinum (100 mg once per rectum № 10) and clotrimazole topically (Table 1. per vagina singly evening № 10).

Table 1.

<table>
<thead>
<tr>
<th>Patients with chronic adnexitis in the exacerbation phase</th>
<th>№</th>
<th>Treatment mode</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>Standard medicinal treatment</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Standard medicinal treatment + «Longidaza» suppositories</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Standard medicinal treatment + «Gepon»</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Standard medicinal treatment + «Longidaza» injections</td>
<td>17</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Donors</td>
<td></td>
<td></td>
<td>22</td>
</tr>
</tbody>
</table>

The second group was included patients (17 women), which put on «Longidaza» in the form of suppositories (1 suppository 3000 per recti 1 times a day for 5 days).

In the third group (18 patients), patients received, in addition to a standard treatment regimen «Gepon» (10 mg per 1 times a day for 5 days).

The fourth group was included patients (17 women), which put on «Longidaza» injections (1 suppository 3000 intramuscularly 1 time per day for 5 days).

All drugs were administered according to the guidelines outlined in the guide «Medicines», «Doctor-register own funds of Russia» (2010) and instructions on the use of drugs.

Laboratory research. Content FNOα, IL-18,
INFa, IL-10, C3 and C4 components of the complement system, factor H was determined in the blood plasma and cervical-vaginal lavage with the help of ProCon reagent kit (LLC «Protein contour», St. Petersburg) by enzyme immunoferment analysis.

Intensity of lipid peroxidation was evaluated on the content in the blood and cervical-vaginal lavage malonic dial [20]. The measured activity of catalase [21], SOD, the concentration of ceruloplasmin-governmental stable metabolites of nitric oxide [22], α1-antitrypsin, α2-macroglobulin and total antioxidant activity of blood serum [23].

Erythrocytes were prepared from 5 ml heparinized blood by the method of E. Beutler with minor modification. Whole blood is asserted twice in 10 mM Na-phosphate buffer (pH 7.4) containing 0.9% sodium chloride and 3% dextran T-500, for 30 minutes at 37 °C. Thereafter, the blood was centrifuged, the supernatant was removed by aspiration. Packed red blood cells were further purified to the chromatographic column through HBs-cellulose.

We determined the total sorption capacity of red blood cells and the sorption capacity of glycocalyx.

On the functional state of erythrocytes as judged by the accumulation of malonic dial and activity of superoxide dismutase.

**Statistical analysis of the results.** Statistical processing of results of research was carried out using non-parametric methods, factor analysis, cluster analysis, and Spearman's rank correlation coefficient. Differences were considered statistically significant with p <0.05.

For immunological parameters were calculated ratio diagnostic value, determined by the formula of immune disorders system by selecting from all the studied parameters of the top three most distinguished level of standards expected level of immune disorders, ranking algorithm largest extent disorders conducted.

Correlation analysis between indices immunometabolic and clinical data, calculated the amount of degrees of correction for each treatment regimen.

**RESULTS OF RESEARCH**

Immune and metabolic disorders in patients with chronic adnexitis in the exacerbation phase before and after standard treatment. The studied parameters of patients with chronic adnexitis in the exacerbation phase in the surveyed group to the treatment of each other do not differ. Patients with chronic adnexitis in the exacerbation phase plasma at admission to hospital revealed incr

Using the standard treatment in patients with chronic adnexitis in the exacerbation phase allowed to reduce, but not to the level of standards, the concentration of IL-18, C3 and C4 of the complement system and reduced levels of IL-10 over 4 pain and factor H (Table 2).

In the cervical-vaginal lavage in women with chronic adnexitis in the exacerbation phase revealed raising FNOα, IL-18, INFa, system components plicements someone, but the decline in the level of secretory IgA, factor H and IL-10 (Table 3). Against the background of standard treatment in patients with chronic adnexitis in the exacerbation phase in the vaginal-cervical lavage is reduced, but not to the level of standards, the concentration of IL-18, INFa and increased levels of IL-10 (Table 3).

### Table 2.

**Cytokines and complement concentration of system components in patients with chronic adnexitis in the exacerbation phase plasma on a background of standard treatment.**

<table>
<thead>
<tr>
<th>Exponents</th>
<th>Units</th>
<th>1 Healthy</th>
<th>2 Patients with chronic adnexitis in the exacerbation phase</th>
<th>3 Before treatment</th>
<th>4 After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>FNOα</td>
<td>pg/ml</td>
<td>1,77±0,08</td>
<td>5,8±0,15 * 1 &lt;sup&gt;1&lt;/sup&gt;</td>
<td>5,48±0,25 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>pg/ml</td>
<td>40,45±2,35</td>
<td>195,8±8,8 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td>150,1±10,35 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>pg/ml</td>
<td>18,5±0,42</td>
<td>3,5±0,2 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td>3,89±0,24 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>INFα</td>
<td>pg/ml</td>
<td>11,5±1,21</td>
<td>28,6±3,4 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td>26,2±2,19 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>pg/ml</td>
<td>106,4±6,9</td>
<td>228,2±7,6 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td>152,48±7,17 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Factor H</td>
<td>ng/ml</td>
<td>13,6±1,31</td>
<td>47,7±1,6 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td>40,01±1,24 * 1 &lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Note. Hereafter the asterisk marked significant differences of average arithmetical (p<0,05); figures close to the star are in relation to that of a group of these differences.
The concentration of cytokines and complement components in patients current with chronic adnexitis in the exacerbation phase in the vaginal-cervical lavage against standard treatment (M ± m).

<table>
<thead>
<tr>
<th>Exponents</th>
<th>Units</th>
<th>1: healthy</th>
<th>2: Patients with chronic adnexitis in the exacerbation phase</th>
<th>3: Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FNOα</td>
<td>pg/ml</td>
<td>1,19±0,08</td>
<td>6,1±0,2</td>
<td>6,65±0,46</td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>pg/ml</td>
<td>2,58±0,13</td>
<td>14,3±0,9</td>
<td>10,91±0,89</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>pg/ml</td>
<td>5,12±0,77</td>
<td>1,8±0,14</td>
<td>3,94±0,57</td>
<td></td>
</tr>
<tr>
<td>INFα</td>
<td>pg/ml</td>
<td>8,37±0,59</td>
<td>14,5±1,1</td>
<td>10,8±1,2</td>
<td></td>
</tr>
<tr>
<td>C₃</td>
<td>mg/dl</td>
<td>24,3±2,9</td>
<td>40,6±1,7</td>
<td>41,55±2,08</td>
<td></td>
</tr>
<tr>
<td>C₄</td>
<td>mg/dl</td>
<td>1,13±0,07</td>
<td>2,5±0,02</td>
<td>2,42±0,11</td>
<td></td>
</tr>
<tr>
<td>Factor H</td>
<td>ng/ml</td>
<td>29,8±2,14</td>
<td>17,3±1,1</td>
<td>18,0±1,21</td>
<td></td>
</tr>
<tr>
<td>sIgA</td>
<td>mg/dl</td>
<td>37,6±1,24</td>
<td>31,8±0,1</td>
<td>31,96±1,09</td>
<td></td>
</tr>
</tbody>
</table>

In the blood plasma of patients with identified chronic adnexitis in the exacerbation phase raising MDA, α₁-AT, α₂-MG, stable metabolite of nitric oxide in reducing CRP during General antioxidantive activity serum, SOD and catalase activity, the concentration of ceruloplasmin (Table 4).

On the background of the standard treatment for this category of patient current plasma malonic dial concentration decreases, CRP, proteolytic ferments, but increased the activity of catalase, SOD, General antioxidantive activity, but not to the level healthy donors (Table 4).

Table 4.

Indicators of metabolic status of patients with chronic adnexitis in the exacerbation phase in the blood on the background of standard treatment (M ± m).

<table>
<thead>
<tr>
<th>Exponents</th>
<th>Units</th>
<th>1: healthy</th>
<th>2: patients with chronic adnexitis in the exacerbation phase</th>
<th>3: Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malonic dial</td>
<td>umol/l</td>
<td>1,98±0,15</td>
<td>4,1±0,08</td>
<td>3,8±0,05</td>
<td></td>
</tr>
<tr>
<td>General antioxidantive activity</td>
<td>%</td>
<td>50,1±0,99</td>
<td>39,3±0,7</td>
<td>43,2±0,56</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>U/ml</td>
<td>12,8±0,99</td>
<td>4,2±0,2</td>
<td>5,93±0,34</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>µkat/L</td>
<td>22,65±1,63</td>
<td>11,4±0,3</td>
<td>12,86±0,28</td>
<td></td>
</tr>
<tr>
<td>ceruloplasmin</td>
<td>mg/dl</td>
<td>33,2±2,05</td>
<td>24,5±1,9</td>
<td>22,0±1,19</td>
<td>58,9±4,11</td>
</tr>
<tr>
<td>CMNO</td>
<td>mg/dl</td>
<td>33,2±2,05</td>
<td>24,5±1,9</td>
<td>22,0±1,19</td>
<td>58,9±4,11</td>
</tr>
<tr>
<td>α₁-AT</td>
<td>mg/dl</td>
<td>80,6±4,3</td>
<td>134,3±3,7</td>
<td>90,88±2,35</td>
<td></td>
</tr>
<tr>
<td>α₂-MG</td>
<td>mg/dl</td>
<td>89,5±2,09</td>
<td>140,4±6,1</td>
<td>113,0±3,48</td>
<td>22,0±1,19</td>
</tr>
<tr>
<td>CRP</td>
<td>mg/dl</td>
<td>1,35±0,33</td>
<td>2,1±0,04</td>
<td>1,73±0,08</td>
<td></td>
</tr>
</tbody>
</table>

In the cervical-vaginal lavage women with chronic adnexitis in the exacerbation phase revealed increased concentration of malonic dial, stable metabolites of azote oxide, α₁-AT, α₂-MG and reducing General antioxidantive activity, the activity of SOD, catalase, the concentration of ceruloplasmin (Table 5).

Table 5.

Indicators of metabolic status of patients with chronic adnexitis in the exacerbation phase in the vaginal-cervical lavage on a background of standard treatment (M ± m).

<table>
<thead>
<tr>
<th>Exponents</th>
<th>Units</th>
<th>1: healthy</th>
<th>2: patients with chronic adnexitis in the exacerbation phase</th>
<th>3: Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malonic dial</td>
<td>umol/l</td>
<td>0,87±0,03</td>
<td>2,1±0,05</td>
<td>1,98±0,08</td>
<td></td>
</tr>
<tr>
<td>General antioxidantive activity</td>
<td>%</td>
<td>15,6±1,12</td>
<td>4,8±0,27</td>
<td>7,46±0,45</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>U/ml</td>
<td>2,07±0,08</td>
<td>0,5±0,05</td>
<td>0,62±0,09</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>µkat/L</td>
<td>3,16±0,09</td>
<td>1,5±0,18</td>
<td>1,14±0,34</td>
<td></td>
</tr>
<tr>
<td>ceruloplasmin</td>
<td>mg/dl</td>
<td>2,48±0,04</td>
<td>0,7±0,06</td>
<td>0,97±0,04</td>
<td></td>
</tr>
<tr>
<td>CMNO</td>
<td>umol/l</td>
<td>0,51±0,03</td>
<td>0,6±0,05</td>
<td>0,46±0,04</td>
<td></td>
</tr>
<tr>
<td>α₁-AT</td>
<td>mg/dl</td>
<td>13,7±0,71</td>
<td>40,0±2,06</td>
<td>15,7±0,98</td>
<td></td>
</tr>
<tr>
<td>α₂-MG</td>
<td>mg/dl</td>
<td>18,09±1,13</td>
<td>25,8±2,09</td>
<td>21,6±3,35</td>
<td></td>
</tr>
</tbody>
</table>
Using a standard treatment regimen in patients with chronic adnexitis in the exacerbation phase possible to normalize the level of α₁-AT, but to correct burdened obstetric history, concentration of ceruloplasmin, without affecting the rest of the indicators changed in the metabolic status (Table 5).

We have further studied the sorption properties of red blood cells and the concentration within them of malonic dial and SOD activity. Thus, in patients with chronic adnexitis in the exacerbation phase in erythrocyte malonic dial concentration increased and reduced on-SOD activity (Figure 1).

![Figure 1](image_url)

In addition, when you receive in this category of patients increased sorption properties of erythrocytes: sorption capacity of erythrocyte and sorptive capacity glycosalys (Figure 1.). Against the background of standard treatment in patients with chronic adnexitis in the exacerbation phase concentration decreases in erythrocyte malonic dial, but not to the level of standards, while other indicators remain at the same level changed (Figure 1).

Comparing the total number of different parameters of normal levels in patients with chronic adnexitis in the exacerbation phase found that if such indicators before treatment was 96.6%, after the standard pharmacotherapy such indicators was 94.2, which is not enough (table 6).

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Standard treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent level of standards before treatment</td>
<td>96.6</td>
</tr>
<tr>
<td>normalized</td>
<td>2.4</td>
</tr>
<tr>
<td>corrected</td>
<td>23.3</td>
</tr>
<tr>
<td>Not changing</td>
<td>70.9</td>
</tr>
<tr>
<td>Excellent level of standards after standard therapy</td>
<td>94.2</td>
</tr>
</tbody>
</table>

**Table 6.**

The effectiveness of standard treatment in patients with chronic adnexitis in the exacerbation phase (% Indicators).

**Immune efficacy of «Gepon» and «Longidaza» in patients with chronic adnexitis in the exacerbation phase.** Using «Longidaza» in the form of suppositories for patients with chronic adnexitis in the exacerbation phase in addition to a standard treatment regimen will normalize the blood concentration of the C3 component of complement system, INFα, reduce, but not to the level of standards, the level FNOα, IL-18, C4 component of the complement system (Table 7).

At the local level in patients with chronic adnexitis in the exacerbation phase against
application «Longidaza» in the form of suppositories to normal levels of IL-18, INFα, C4 component of the complement system, of sIgA, corrected, but not to the level of standards, the concentration of FNOα, factor H (Table 8).

Appointment «Longidaza» in the form of suppositories patients with chronic adnexitis in the exacerbation phase additory to the standard treatment regimen helped normalize blood concentration of ceruloplasmin, CRP increase, but not to the level of standards, SOD activity, catalase, α2-MG (Table 7).

Women with chronic adnexitis in the exacerbation phase against application «Longidaza» in the form of suppositories in the vaginal-cervical lavage normalized level of malonic dial, SMNO proteolytic enzymes, corrected, but not to the level of standards activity catalase, SOD and the level of ceruloplasmin (Table 8).

Using «Gepon» in patients with chronic adnexitis in the exacerbation phase in addition to the standard treatment regimen helped correct the concentration in the blood FNOα, IL-18, INFα, but further increases the concentration of component B tem Complement: C3 and complement C4 component (Table 7).

At the local level in patients with chronic adnexitis in the exacerbation phase against application «Gepon» in the form of injections corrected FNOα level and IL-18 (Table 8).

Appointment «Longidaza» in the form of injections patients with additional chronic adnexitis in the exacerbation phase to the standard treatment regimen helped normalize blood concentration of ceruloplasmin, α1-AT, CRP increase, but not to the level of standards are SOD and catalase, the concentration of α2- MG (Table 7). Women with chronic adnexitis in the exacerbation phase against application «Longidaza» in the form of injections in the vaginal-cervical lavage normalized level of malonic dial, General antioxidative activity, SMNO and α2-MG, corrected, but not to the level of standards, catalase activity and the level of α1-AT (Table 8). The use of «Gepon» corrects inside the erythrocytes malonic dial level and sorption capacity of erythrocyte, «Longidaza» in the form of suppositories further sorptive capacity glycoalyx, while the use of «Longidaza» in the form of injections normalize sorption properties of red blood cells (Figure 2).
### Table 7.

<table>
<thead>
<tr>
<th>Exponents</th>
<th>Units</th>
<th>healthy</th>
<th>After treatment (M ± m)</th>
<th>patients with chronic adenexitis in the exacerbation phase</th>
<th>After treatment (M ± m)</th>
<th>Standard treatment + «Gepon»</th>
<th>After treatment (M ± m)</th>
<th>Standard treatment + «Longidaza» injections</th>
<th>After treatment (M ± m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FNOα</td>
<td>pg/ml</td>
<td>1,77±0,08</td>
<td>6,0±0,15 *1</td>
<td>2,51±0,06 *1,2</td>
<td>5,9±0,15 *1</td>
<td>3,59±0,15 *1,4</td>
<td>5,8±0,15 *1</td>
<td>1,79±0,07 *6</td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>pg/ml</td>
<td>40,45±2,35</td>
<td>198,4±9,8 *1</td>
<td>137,73±4,3,3 *1</td>
<td>190,7±8,7 *1,2</td>
<td>70,17±2,58 *1,4</td>
<td>191,7±10,2 *1</td>
<td>53,1±2,4 *1,6</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>pg/ml</td>
<td>18,5±0,42</td>
<td>3,7±0,2 *1</td>
<td>3,35±0,15 *1</td>
<td>3,6±0,3 *1</td>
<td>3,64±0,11 *1</td>
<td>3,3±0,2 *1</td>
<td>9,8±1,09 *1,6</td>
<td></td>
</tr>
<tr>
<td>INFa</td>
<td>pg/ml</td>
<td>11,5±1,21</td>
<td>27,5±2,5 *1</td>
<td>12,5±1,96 *2</td>
<td>28,8±3,1 *1</td>
<td>20,4±2,3 *1,2</td>
<td>29,9±3,1 *1</td>
<td>12,0±2,14 *6</td>
<td></td>
</tr>
<tr>
<td>CR1</td>
<td>mg/dl</td>
<td>106,4±6,9</td>
<td>246,1±12,1 *1</td>
<td>112,9±3,2 *1</td>
<td>212,8±9,6 *1</td>
<td>175,64±4,04 *1,4</td>
<td>230,8±10,5 *1</td>
<td>115,47±2,18 *6</td>
<td></td>
</tr>
<tr>
<td>CR2</td>
<td>mg/dl</td>
<td>13,6±1,31</td>
<td>49,1±1,8 *1</td>
<td>25,92±0,88 *1</td>
<td>43,5±1,7 *1</td>
<td>56,06±1,7 *1,4</td>
<td>50,6±1,3 *1</td>
<td>14,83±1,45 *6</td>
<td></td>
</tr>
<tr>
<td>Factor H</td>
<td>ng/ml</td>
<td>41,3±3,3</td>
<td>25,1±1,8 *1</td>
<td>32,8±4,7 *1,2</td>
<td>24,2±1,4 *1</td>
<td>30,2±2,51 *1,2</td>
<td>23,2±1,9 *1</td>
<td>43,5±4,06 *6</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>umol/l</td>
<td>1,98±0,15</td>
<td>4,3±0,09 *1</td>
<td>4,0±0,07 *1</td>
<td>3,62±0,16 *1</td>
<td>4,4±0,09 *1</td>
<td>4,2±0,07 *1</td>
<td>2,43±0,07 *6</td>
<td></td>
</tr>
<tr>
<td>OAA</td>
<td>%</td>
<td>50,1±0,99</td>
<td>39,5±0,8 *1</td>
<td>38,8±0,8 *1</td>
<td>42,59±0,66 *1,4</td>
<td>40,1±0,8 *1</td>
<td>44,45±0,69 *1,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>U/ml</td>
<td>12,8±0,99</td>
<td>12,8±0,99</td>
<td>4,5±0,3 *1</td>
<td>4,1±0,3 *1</td>
<td>9,06±0,89 *1,4</td>
<td>4,1±0,3 *1</td>
<td>8,13±0,24 *6</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>µkat/L</td>
<td>22,65±1,63</td>
<td>22,65±1,63</td>
<td>11,1±0,4 *1</td>
<td>10,9±0,3 *1</td>
<td>14,23±0,17 *1,4</td>
<td>11,3±0,3 *1</td>
<td>18,46±0,19 *1,6</td>
<td></td>
</tr>
<tr>
<td>ceruloplasmin</td>
<td>mg/dl</td>
<td>33,2±2,05</td>
<td>33,2±2,05</td>
<td>24,8±1,8 *1</td>
<td>23,8±1,8 *1</td>
<td>25,36±1,29 *1</td>
<td>22,8±1,9 *1</td>
<td>31,06±3,5 *6</td>
<td></td>
</tr>
<tr>
<td>CMKo</td>
<td>umol/l</td>
<td>3,68±0,21</td>
<td>4,2±0,2 *1</td>
<td>4,1±0,2 *1</td>
<td>2,6±0,16 *1</td>
<td>4,2±0,2 *1</td>
<td>2,8±0,11 *1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1-AT</td>
<td>mg/dl</td>
<td>80,6±4,3</td>
<td>80,6±4,3</td>
<td>141,2±3,8 *1</td>
<td>131,8±3,8 *1</td>
<td>91,2±2,5 *1,4</td>
<td>131,4±3,6 *1</td>
<td>81,3±1,87 *6</td>
<td></td>
</tr>
<tr>
<td>α2-MG</td>
<td>mg/dl</td>
<td>89,5±2,09</td>
<td>89,5±2,09</td>
<td>141,8±6,2 *1</td>
<td>135,9±6,1 *1</td>
<td>136,8±3,66 *1</td>
<td>134,6±6,2 *1</td>
<td>96,4±2,56 *1,6</td>
<td></td>
</tr>
<tr>
<td>CRB</td>
<td>mg/dl</td>
<td>1,35±0,33</td>
<td>1,35±0,33</td>
<td>2,2±0,05 *1</td>
<td>2,3±0,08 *1</td>
<td>1,91±0,09 *1</td>
<td>2,0±0,04 *1</td>
<td>1,52±0,03 *9</td>
<td></td>
</tr>
</tbody>
</table>
**Immune and metabolic status at the local level in patients with chronic adnexitis in the exacerbation phase: the therapy (M ± m).**

<table>
<thead>
<tr>
<th>Units</th>
<th>Healthy patients with chronic adnexitis in the exacerbation phase</th>
<th>Standard treatment + «Longidaza» suppositories</th>
<th>Standard treatment + «Gepon»</th>
<th>Standard treatment + «Longidaza» injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN0α pg/ml</td>
<td>1,19±0,08</td>
<td>6,5±0,3 1</td>
<td>2,52±0,39 1,4</td>
<td>6,3±0,2 1</td>
</tr>
<tr>
<td>IL-18 pg/ml</td>
<td>2,58±0,13</td>
<td>12,8±0,8 1</td>
<td>2,69±0,33 2,4</td>
<td>14,5±0,9 1</td>
</tr>
<tr>
<td>LH0α pg/ml</td>
<td>5,12±0,77</td>
<td>1,9±0,1 1</td>
<td>3,39±0,22 1,4</td>
<td>1,7±0,1 1</td>
</tr>
<tr>
<td>C6 mg/dl</td>
<td>24,3±2,9</td>
<td>36,6±1,9 1</td>
<td>40,68±3,76 1,4</td>
<td>46,7±1,6 1</td>
</tr>
<tr>
<td>C4 mg/dl</td>
<td>1,13±0,07</td>
<td>2,2±0,03 1</td>
<td>2,6±0,02 1</td>
<td>2,51±0,47 1</td>
</tr>
<tr>
<td>Factor H ng/ml</td>
<td>29,8±2,14</td>
<td>16,1±1,0 1</td>
<td>38,76±3,02 1</td>
<td>37,9±0,1 1</td>
</tr>
<tr>
<td>sIgA mg/dl</td>
<td>37,6±1,24</td>
<td>30,2±0,4 1</td>
<td>38,76±3,02 1</td>
<td>37,9±0,1 1</td>
</tr>
<tr>
<td>MDA umol/l</td>
<td>0,87±0,03</td>
<td>0,91±0,03 1</td>
<td>2,2±0,06 1</td>
<td>2,05±0,05 1</td>
</tr>
<tr>
<td>OAA %</td>
<td>15,6±1,12</td>
<td>4,4±3,1 1</td>
<td>13,3±0,49 1,4</td>
<td>4,9±0,25 1</td>
</tr>
<tr>
<td>SOD U/ml</td>
<td>2,07±0,08</td>
<td>0,6±0,05 1</td>
<td>1,73±0,07 1,4</td>
<td>0,4±0,04 1</td>
</tr>
<tr>
<td>Catalase μkat/L</td>
<td>3,16±0,09</td>
<td>1,4±0,1 1</td>
<td>2,86±0,12 1,4</td>
<td>1,6±0,15 1</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>2,48±0,04</td>
<td>0,8±0,07 1</td>
<td>2,1±0,04 1,4</td>
<td>0,6±0,07 1</td>
</tr>
<tr>
<td>CM90 umol/l</td>
<td>0,51±0,03</td>
<td>0,6±0,08 1</td>
<td>0,45±0,04 1,4</td>
<td>0,7±0,06 1</td>
</tr>
<tr>
<td>α2-AT mg/dl</td>
<td>13,7±0,71</td>
<td>38,6±2,2 1</td>
<td>12,97±0,63 2,4</td>
<td>40,2±2,05 1</td>
</tr>
<tr>
<td>α2-MG mg/dl</td>
<td>18,09±1,13</td>
<td>26,2±2,2 1</td>
<td>17,72±1,36 1</td>
<td>24,1±2,1 1</td>
</tr>
</tbody>
</table>
Comparative clinical and laboratory efficiency «Gepon» and another forms «Longidaza» in patients with chronic adnexitis.

Using «Gepon» normalizes 16.6% of the indicators, and the use of «Longidaza» in the form of suppositories - 28.6%, which reduced the number of indicators other than the level of the norm to 77.6 and 65.6%. At the same time the using of «Longidaza» in the form of injections in patients with chronic adnexitis in the exacerbation phase allowed normalized larger number of indicators, making lower the number of modify to 58.8% (Table 9).

**Table 9.**

<table>
<thead>
<tr>
<th>Exponents (%) from the total exponents</th>
<th>Standard therapy + «Gepon»</th>
<th>Standard therapy + «Longidaza» suppositories</th>
<th>Standard therapy + «Longidaza» injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent on the level of the standard rate of pharmacotherapy</td>
<td>94.2</td>
<td>28.6</td>
<td>35.4</td>
</tr>
<tr>
<td>Normalized</td>
<td>16.6</td>
<td>28.6</td>
<td>35.2</td>
</tr>
<tr>
<td>Corrected</td>
<td>25.6</td>
<td>36.4</td>
<td>23.6</td>
</tr>
<tr>
<td>Unaltered</td>
<td>52.0</td>
<td>29.2</td>
<td>58.8</td>
</tr>
<tr>
<td>Excellent level of standards after additional pharmacological correction</td>
<td>77.6</td>
<td>65.6</td>
<td>58.8</td>
</tr>
</tbody>
</table>

In determining its own corrective effects of treatment, regimens compared with standard conservative therapy found that the practically all indicators of immune status of all these diagram effectiveness standard, as the average degree of correction in almost all performance parameters are proved positive.

**Figure 2.** Condition of the metabolic status of erythrocytes in patients with chronic adnexitis in the exacerbation phase on a background of therapies

For example, 36.8% of patients after standard treatment with chronic adnexitis in the exacerbation phase has pain, 15% - dysmenorrhea, 36.8 - increase the basal rate during menstruation, at 32.2% there is an increase of leukocytes in the smears (Table. 10).
Clinical effectiveness of different treatment regimens of patients with chronic adenexitis in the exacerbation phase (% of patients).

<table>
<thead>
<tr>
<th>№</th>
<th>Treatment</th>
<th>Relapses exacerbations (&gt; 1 during the year)</th>
<th>Increasing leukocytes in the smears</th>
<th>Increasing basal temperature during menstruation</th>
<th>Pain syndrome</th>
<th>Dysmenorrhea</th>
<th>The secretory dysfunction (whites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Standard treatment (ST)</td>
<td>32.2</td>
<td>32.2</td>
<td>36.8</td>
<td>36.8</td>
<td>15.0</td>
<td>40.0</td>
</tr>
<tr>
<td>2.</td>
<td>ST + «Gepon»</td>
<td>12.5</td>
<td>*1</td>
<td>*1</td>
<td>*1</td>
<td>*1</td>
<td>*1</td>
</tr>
<tr>
<td>3.</td>
<td>ST + «Longidaza» suppositories</td>
<td>6.7</td>
<td>*1</td>
<td>*1</td>
<td>*1</td>
<td>*1</td>
<td>*1</td>
</tr>
<tr>
<td>4.</td>
<td>ST + «Longidaza» injections</td>
<td>*1.3</td>
<td>13.3</td>
<td>*1.3</td>
<td>*1.2</td>
<td>*1.2</td>
<td>*1.2</td>
</tr>
</tbody>
</table>

The maximum decrease in clinical manifestations observed after the application of an additional «Longidaza» in the form of injections, after the appointment of the drug were no relapses of diseases in 13.3% of patients was observed and dysmenorrhea, increase in white blood cells in the smears and increase basal temperature.

With multiple correlation matrix the Spearman between exponent of immune, metabolic status and clinical manifestations were significant, both positive and negative connections, testified of the interdependence of laboratory and clinical long-exponent.

A number of indicators have the greatest number of these relation: concentration in the blood plasma FNOα, C3 component complement system, α1-antitrypsin, and cervical-vaginal lavage - the activity of superoxide dismutase.

In patients with chronic adenexitis in the exacerbation phase has been a violation of a number of indicators of immune status, as in system, and at the local level, at the same time it is carried out the standard pharmacological correction in these patients does not allow fully resolve as the immune, so and metabolic status, which dictates the desirability of research and testing in clinical trials of more pharmacological agents and methods immunorehabilitation patients with chronic adenexitis in the exacerbation phase, in this connection, we have established Immune efficacy of «Gepon» and «Longidaza» in patients with chronic chronic adenexitis in the exacerbation phase. In the end, we used the drug «Gepon» and «Longidaza» in two dosage forms - suppositories and injectable form.

Use in addition to standard pharmacotherapy chronic adenexitis in the exacerbation phase «Gepon» helped normalize blood levels only α1-antitrypsin, while the use of «Longidaza» in the form of suppositories further normalizes the concentration of CRP, INFα and C3 component of the complement system. This is the use of an additional «Longidaza» injection enabled normalized larger number of indicators at the system level.

The use of «Gepon» does not have a normalizing effect on the changed indicators were in vaginal-cervical lavage women with chronic adenexitis in the exacerbation phase, whereas the use of «Longidaza» as suppositories normalizes studied indices of metabolic status, whereas injectable normalizes a level of α2-macroglobulin and INFα.

The use of «Gepon» corrects inside the erythrocytes malonic dial level and sorption capacity of erythrocyte, «Longidaza» in the form of suppositories - further sorptive capacity glycoalyx, while the use of «Longidaza» in the form of injections normalize sorption properties of erythrocytes.

Thus, on the basis of the research we can recommend to use in practical public health sketch
pharmacologists immuno-metabolic rehabilitation in patients with chronic adnexitis in the exacerbation phase using «Longidaza» in the form of injections. Less effective, it corrects the disturbed immune parameters and oxidative status of the treatment regimen with the use of this drug in the form of suppositories.

**RECOMMENDATION**

Taking into account the results expressed in the form of the article, we can, but to formulate the recommendations and prospects of further development of the theme:

- In order to mitigate immune and oxidant disturbances in patients with chronic adnexitis in the exacerbation phase in the form of injection (3000 IU intramuscularly 1 time per day for 5 days) is possible the use of exacerbation «Longidaza».
- To obtain objective facts on the severity of Immune disorders in patients with chronic adnexitis in the exacerbation phase usefully be measured in the blood plasma concentration FNOα, C3-component system someone plementa, α1-antitrypsin, and cervical-vaginal lavage - the concentration of IL-18, the activity of superoxide dismutase.
- Use in the educational process of medical universities the knowledge about the reacting immunocorrective and antioxidant effects «Longidaza» and «Gepon» in terms of exacerbation of chronic adnexitis.

**CONCLUSIONS**

After the standard treatment in patients with chronic adnexitis in the exacerbation phase has not reached the level of healthy donor concentration in plasma IL-18, C3, C4 components of the complement system, α1-antitrypsin, α2-macroglobulin. C-reactive protein in vaginal-cervical lavage concentrations of stable metabolites of nitric oxide, IL-10 and α1-antitrypsin, α2-macroglobulin and endoglobin concentrations of malonic dial.

Use in addition to standard pharmacotherapy of chronic adnexitis «Gepon» (in a dosage of 10 mg per 1 times a day for 5 days) helped further to normalize at B level α1-antitrypsin, inside red blood cells to correct the level of malonic dial and total sorption capacity blood cells without significant effects on performance at the local level.

Appointment of patients with chronic adnexitis in the exacerbation phase, in addition to the standard treatment regimen, «Longidaza» in the form of suppositories (in a dosage of 1 suppository 3000 per recti 1 times a day for 5 days) complement normalizes blood plasma concentration of C-reactive protein, INFα and C3 component of the complement system, sorption capacity glicocalyx red blood cells, and vaginal-cervical lavage almost all the studied indicators of metabolic status.

Using an additional «Longidaza» injection (in a dosage of 1 suppository 3000 intramuscularly 1 time per day for 5 days) in patients with chronic adnexitis in the exacerbation phase helped normalize the greater number of indicators at the system level: sorption properties of red blood cells, and at the local level only level α2-macroglobulin and INFα.

Using «Gepon» normalizes 16.6% of the indicators (2.8 times more effective than standard therapy), and the use of «Longidaza» in the form of suppositories - 28.6% (4.9 times more effective than standard therapy), which reduced the number of indicators other than the level of the norm to 77.6 and 65.6%. At the same time the using of «Longidaza» in the form of injections in patients with chronic adnexitis in the exacerbation phase allowed normalized larger number of indicators, making lower the number of modify to 58.8% (6.1 times more effective than standard therapy).

**References**


THE ANALYSIS OF PREFERENCES OF OBSTETRICIANS-GYNECOLOGISTS AND THERAPISTS OF BELGOROD REGION IN HEARTBURN AND CONSTIPATION TREATMENT IN PREGNANT WOMEN IN COMPARISON WITH ALL-RUSSIAN DATA

Summary. One of the most frequent accompanying pathologies during pregnancy – digestive tract diseases. The work purpose - to analyse the choice of medicinal therapy among doctors at heartburn and constipation treatment during pregnancy. Materials and methods - the analysis of anonymous questioning within carrying out the second stage of the All-Russian pharmacoepidemiological research "Epidemiology of medicines use in pregnant women” which was carried out from February to April, 2015. The comparison of the received results with results of questioning of doctors of Belgorod region was executed on the basis of received data. Results and discussion. About a half of doctors chooses for heartburn treatment in pregnant women antacids medicines, and also inhibitors of a proton pomp (omeprazole). To 10% of doctors prescribe insufficiently studied blockers of H2-histamine receptors. In constipation therapy more than 60% of doctors voted for salt laxative (senna and macrogol). Less than 11% of doctors prescribed to patients unsafe medicines of lactulose. Conclusion. The analysis of doctors’ answers on tactics of medicinal therapy purpose at heartburn and constipation during pregnancy has shown that more than 68% of the practicing experts appoint the therapy, based on rational use of medicines.

Keywords: pregnancy, heartburn, constipation, lactulose, antacids medicines, inhibitors of a proton pomp, rational pharmacotherapy.
The purpose of GERD treatment during the gestational period is the maximum use of agents of non-drug therapy, especially in the first trimesters of pregnancy [1, 4, 6]. Such actions are referred to change of lifestyle of a pregnant woman and feeding behavior. After meal it is necessary to avoid the compelled position of a body at which heartburn appear or increase – forward inclination of body, horizontal position, physical activity with a strain of abdominal muscles, wearing of tight belt. During sleep it is better to raise the head extremity of a bed by 10-15 degrees.

Concerning a diet at heartburn and GERD it is necessary to adhere to fractional meal of small portions (to 6-7 times a day) [5, 6, 7, 8], to avoid products which irritate gastric mucosa and gullet – fried and spicy dishes, smoked products, row vegetables (especially which contain rough cellulose – a white cabbage, a garden radish, a radish, garlic), sour fruit and juice, carbonated drinks, black bread, chocolate, chocolates. Optimum impact on a mucous membrane is exerted by steamed food or dishes made in oven, baked vegetables and fruit, low-fat sort of meat or fish, milk, cream, cottage cheese. These products belong to "natural antacid". Such simple actions help to avoid purpose of medicaments therapy in 60%-70% of cases of heartburn occur. Also in some situations it is enough to drink several drinks of water of room temperature for prevention of an eructation or heartburn [7, 9].

However, when these methods become insufficiently, for improvement of quality of pregnant woman life it is necessary to use medical preparations [7, 9, 11]. Traditionally for symptoms of heartburn reduction three types of drugs are applied which influence variously on a heartburn pathogenesis – reduce hydrochloric acid production in a stomach (the inhibitors of a proton pomp (IPP) and H2-gistaminoblocers (H2-GB)) and neutralize already emitted hydrochloric acid (antacid drugs). IPP (omeprazole) (category B on medicines classification of Food and Drug Administration (FDA) of USA) and antacid drugs are referred to the resolved drugs during pregnancy as the least soak up in a systemic blood stream through a mucosa of an esophagus and a stomach [8, 12].

One more widespread pathology during pregnancy is constipation – a chronic delay of defecation with intestines depletion less than three times a week [13]. The constipation is followed by such feelings as feeling of incomplete intestines depletion, the dense and small quantity of fecal masses, discomfort in a stomach, nausea, a loss of appetite, the suppressed mood. Frequency of occurrence of constipation at women during a gestation is caused by influence of hormones, progesterone in particular, on contractive activity of a large intestine which leads to lowering of physical activity of a thick gut. Reduction of level of a motilin in the II-III trimester of pregnancy, mechanical impaction of a large intestine by increased uterus are also impact on constipation appearance [13, 14, 15].

Emergence of constipation leads to a microbial content of a thick gut, penetration of pathogenic flora into a vagina and to the ascending infection of a Belgorod Region. The delay of the dejection can lead to premature discharge of amniotic fluid, threat of termination of pregnancy, inflammatory diseases of a mucous membrane of a uterus [14, 15, 16].

That’s why it should be paid special attention to therapy of constipation during pregnancy. In most cases it turns out to cope with constipation during a gestation by non-drug ways – to increase physical activity, to diversify a diet with the rich fibers food (bran, dried apricots, prunes, lactic products, a white cabbage, beetroot, tomatoes, marrows) [13, 16]. It is necessary to limit the binding and locking products – white bread, strong black and green tea, coffee, chocolate, farinaceous dishes [15, 17].

When non-drug therapy becomes inefficient, it is necessary to resort to pharmaceutical medicines which are everywhere used during locks. The most effective, safe and reliable agent during a constipation in pregnant women is lactulose [16, 17, 18]. Being osmosaline laxative, the lactulose works as systemic blood stream path, to avoid microbial content of a thick gut, increases osmotic pressure and by that promotes water inflow in a gut that leads to dejection softening [19, 20].

In view of prevalence of GERD and constipation, its social significance and influence on quality of pregnant women life, a large number of complications, it become relevant to carry out a pharmacoepidemiological survey with the purpose of detection of doctors’ preferences in choice of the main medicines of heartburn and constipation treatment in pregnant women.

**Work purpose:** To analyse the choice of AMT of doctors of Belgorod region of heartburn and constipation treatment in pregnant women. To compare data with the results received as a result of questioning which was carried out from February to April, 2015 in 4 federal districts of the Russian Federation – Central, Privolzhskom, Ural and Far-Eastern.

**Materials and methods:** At the heart of this research is the method of anonymous questioning within carrying out the second stage of the All-Russian pharmacoepidemiological research "Epidemiology of medicines use in pregnant women" which was carried...
out from February to April, 2015. In the All-Russian pharmacoepidemiological research (ARR) 1066 questionnaires were analyzed from which 734 obstetricians-gynecologists and 332 therapists [21, 22].

Across Belgorod Region (BR) in questioning have participated: 94 doctors (28.7% of stationary and 69.1% of a polyclinic profile, p < 0.01) from which 77 (81.9%) obstetricians-gynecologists, 17 (18.1%) therapists (p<0.0001), with the general length of work less than 5 years - 21.3% of doctors, 5-10 years – 26.6%, 10-20 years of work – 20.2% of doctors and more than 20 years – 26.6%. Questioning was carried out on the basis of women’s consultation clinic, polyclinics and maternity home of Belgorod, and also in the central regional hospital.

The data received as a result of poll were entered and processed by Microsoft Exel.

**Main part:**
For definition of doctors’ preferences at heartburn treatment the list of the most often used medicines was presented in the questionnaire:
1. Antacids.
2. H2 - GB (ranitidine, famotidine).
3. IPP (omeprazole).
4. Other.

**Comparison of tactics of heartburn treatment in pregnant women among doctors of Belgorod Region and the All-Russian research.**

<table>
<thead>
<tr>
<th>Medicines</th>
<th>Doctors of BR</th>
<th></th>
<th>Doctors of ARR (n=1066)</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=94</td>
<td>%</td>
<td>N=1066</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Antacids</td>
<td>69</td>
<td>73.4</td>
<td>804</td>
<td>75.4</td>
<td>0.664</td>
</tr>
<tr>
<td>IPP (omeprazole)</td>
<td>4</td>
<td>4.3</td>
<td>71</td>
<td>6.7</td>
<td>0.363</td>
</tr>
<tr>
<td>H2- GB (ranitidine, famotidine)</td>
<td>6</td>
<td>7.8</td>
<td>66</td>
<td>6.2</td>
<td>0.941</td>
</tr>
<tr>
<td>Don’t prescribe pharmacotherapy</td>
<td>8</td>
<td>8.5</td>
<td>167</td>
<td>15.7</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Note: IPP-inhibitors of a proton pump, H2-GB – H2-gistaminoblokatory, BR – Belgorod region, ARR – All-Russian research, p – the Chi-square according to Pearson.

For definition of doctors’ preferences at constipation treatment in women in the period of a gestation, the following possible answers have been offered:
1. Lactulose.
2. Senna preparations (Senna, Senade, Regulaks).
3. Macrogol.

Also such possible answers have been provided as "I don't prescribe" and "others", with an opportunity to enter the most preferred medicine.

Lactulose, as the depletive which is most suitable for pregnant women (safe and effective), had been chosen by 68.1% of doctors of BR (67.5% of obstetricians-gynecologists and 70.6% of therapists, p =0.806) and 75.0% of doctors of ARR (p =0.144).

5. Don’t prescribe.

Based on clinical references, antacids as the main agent at heartburn treatment in pregnant women had been chosen by 73.4% of doctors of BR (77.9% of obstetricians-gynecologists and 52.9% of therapists, p =0.03) and 75.4% of doctors of ARR (p =0.664).

Inhibitors of a proton pomp, omeprazole in particular, as the medicine of the second line applied in pregnant women, and belonging to the category B on FDA, had been chosen only by obstetricians-gynecologists of BR (5.2%) and 6.7% of doctors of ARR (p =0.363).

H2-GB (ranitidine, famotidine), which is not recommended for application for pregnant women because of the adverse influences on a condition of a fetus, had been chosen only by obstetricians-gynecologists of BR (7.8%) and doctors of the All-Russian research (6.2%), p =0.941.

8.5% of doctors of BR (5.2% of obstetricians-gynecologists and 23.5% of therapists, p =0.014) and 15.7% of doctors of ARR (p =0.063) had decided to refuse from therapy prescribing to the patients. Comparison of tactics of heartburn treatment in pregnant women among doctors of BR and ARR is presented in Table.

Senna preparations, which are not recommended for application during pregnancy because of possible risks of termination of pregnancy or premature birth, development of a hyponatremia or hypokalemia, had been chosen by 7.5% of experts from BR (7.8% of obstetricians-gynecologists and 5.9% of therapists, p =0.786) and 8.6% of doctors of ARR (p =0.694).

Macrogol, the effective but still insufficiently studied during pregnancy medicine, had been chosen by 6.4% of doctors of BR (6.5% of obstetricians-gynecologists and 5.9% of therapists, p =0.926) and 9.9% of doctors of ARR (p =0.262) [22].

7.5% of doctors of BR (3.9% of obstetricians and 23.5% of therapists, p =0.005) and 11.5% of doctors of ARR (p =0.228) had decided not to prescribe medicines at a constipation in pregnant women.
Among the offered own options of treatment candles with glycerine (locally) were prevalent (6.4% of doctors of BR and 2.3% of doctors of ARR). It also should be noted the special option of constipation treatment during pregnancy by the medicine "Dyufaston" which was written in the questionnaires by 2 obstetricians-gynecologists of BR.

Comparison of tactics of constipation treatment in pregnant women among doctors of BR and ARR is presented in the Picture.

![Frequency of assignment of medicines in case of constipation in pregnant women among obstetricians-gynecologists and therapists of Belgorod region and the Russian Federation.](image)

**Figure.** Frequency of assignment of medicines in case of constipation in pregnant women among obstetricians-gynecologists and therapists of Belgorod region and the Russian Federation.

**Conclusion.**

According to the carried-out questioning, about a half of doctors of Belgorod region and the All-Russian research appoint rational pharmacotherapy at GERB treatment in pregnant women. Antacids as medicine of the first line have chosen more than a half of doctors of BR and ARR, $p = 0.664$. Less than 10% of doctors of various profile have chosen IPP ($p = 0.363$).

Regardless of national clinical references, canons of a rational pharmacotherapy during pregnancy, doctors of BR and ARR (7.8% and 6.2% respectively) decided to prescribe to patients H2-GB which have low evidential base of use for women in the period of a gestation, therefore are potentially dangerous to a fetus.

It is paid much attention to constipation treatment during pregnancy because of high frequency of occurrence of this pathology among women of the gestational period. Accurately implement clinical recommendations and assign salt laxatives, lactulose in particular, more than a half of doctors of Belgorod region and the All-Russian research, $p = 0.144$.

About 10% of doctors have chosen unsafe or isn't final researched in pregnant medicines macrogol and senna medicines. Also, among doctors of Belgorod region and the All-Russian research prevailed not really effective way of solving the problem of constipation – locally application of candles with glycerine, $p = 0.002$.

**Corollary:**

According to questioning, more than 84% of obstetricians-gynecologists and therapists of Belgorod region and the All-Russian research conduct treatment of heartburn and constipations in pregnant women independently. The analysis of answers of doctors of BR and ARR on tactics of prescription of medicines showed that more than 68% of the practicing experts prescribe therapy, based on clinical references and a rational pharmacotherapy. Results of questioning showed once again relevance of a problem of adequate therapy of women during pregnancy at the accompanying pathology.

**References**


5. Vladimir Ivashkin and A. Sheptulin, Diagnosis and treatment of gastroesophageal reflux disease: A guide for physicians (Moscow: 2005), 30. [Full text]


20. N. Chuhareva et al., Results nationwide pharmacoepidemiological study «Epidemiology of drugs during pregnancy” (the second stage),” (paper presented at the XVI All-Russian Scientific Forum “Mother and Child», Moscow, http://elibrary.ru/item.asp?id=26071566 [eLIBRARY]


Abstract. Psychiatric disorders in patients, who suffer from chronic somatic diseases for a long time, can be grouped into one category called psychosomatic pathology. Co-morbid – psychosomatic – disorders are considered to be predictors of unfavourable prognosis significantly aggravating patients’ condition, quality of life and professional activity. It is important to timely diagnose and perform pharmacological correction of affective pathology. Special attention should be paid to patients, whose profession is associated with the operational activity. 110 railroad workers admitted to the in-patient department of the Non-state Health Care Facility “RoadHospital the Station Voronezh-1 of JSC “Russian Railways” with the diagnosis “Chronic pancreatitis, recurrent” were examined in the study. On admission all patients were questioned using specialized diagnostic scales and questionnaires aimed at revealing of anxiodepressive disorders. The authors have found out negative impact of anxiodepressive disorders on the course of chronic pancreatitis with the development of stable pain syndrome, gastro-intestinal disorders, resistance to the performed pharmacotherapy, and decrease of reaction rate to presented stimuli. Examination of the patients suffering from chronic pancreatitis demonstrated that patients with MADD had more severe course of the disease. This manifested in more intensive pain syndrome, apparent symptoms of nausea, bitter taste in the mouth and diarrhea; these symptoms exceeded number of similar complaints in patients with chronic pancreatitis without MADD in 1.6 – 2.1 times. Tranquilizer “Adaptol” and anxiolytic “Afobazol” in combination with basic therapeutical medications efficiently eliminate gastroenterological and anxiety symptoms. However, “Adaptol” decreases rate of visual-motor reactions, whereas “Afobazol”, on the contrary, increases reaction rate to presented stimuli.

Key words: chronic pancreatitis, anxiodepressive disorders, co-morbid pathology, psychotropic therapy, Adaptol, Afobazol, railroad workers.

Introduction. Psycho-somatic disorder is considered to be a psychogenically conditioned pathological state manifesting as an acute somatic pathology, violation of the internal body functions and physiological systems, such as the digestive, respiratory, circulatory, urinary system; this results in a specific complex of symptoms representing body reaction on the development of pathological disorders [11].

Combination of psychic and somatic violations is a characteristic feature of psycho-somatic disorders. Fundamental principle of this branch of medicine is an idea that any disease is not restricted by the clinical symptoms only (nausea, vomiting, abdominal pain, diarrhea etc.), but is manifested at several levels: emotional – a disease causes certain emotions and feelings; cognitive – comprehension of a disease; integrative – the way how an individual perceives, evaluates his condition and sees himself in the existing situation [2, 3].

The most common psychic diseases appear to be anxiety and depressive disorders; their total amount in general medical practice is approaching to 50% [3]. In practice it is very difficult to differentiate one condition from another (co-morbidity is 40 – 80%); in such cases they give evidence of mixed anxiety-depressive disorders (MADD) [5, 6, 7].

Affective disorders developing under gastrointestinal (GI) pathology are of special attention. According to various data, digestive system disorders having psychogenic origin are recorded in 30 – 70% of patients admitted to the in-patient gastroenterology departments [8. 9]. In spite of the large amount of research studies devoted to the investigation of psychosomatics in patients with GI disorders, there are comparatively few data indicating to MADD and chronic pancreatitis co-morbidity. Only a small number of works demonstrate interrelations of psychological manifestations and peculiarities of the course of pancreatic disease (severity, pain intensity, morphological changes) [110, 11, 12, 13].

Development and clinical manifestations of MADD and chronic pancreatitis are pathogenically interrelated. GABAergic and serotonergic systems are considered to be main components of pathogenesis. Reduce of GABAergic activity provides appearance of gastroenterological panic attacks, which are manifested as discomfort in the epigastric region, nausea, rectal reflexes [14]. Serotonin appears to be an important element of gastrointestinal disorders associated with inflammation; this is connected with the violation of the innervations activity [15, 16]. When the amount of serotonin reduces, pain sensitivity increases, and even slight irritation causes apparent pain syndrome [17]. Influence of neurotransmitters on the extramural nerve endings of the gland tissue providing transmission of sensor information to the central nervous system is intensified under the inflammatory reaction. Intensification of their stimulation is associated with nauseous sensation and intestinal dysmotility [18].

Anxiety and depression aggravate the course of somatic pathology, result in delayed onset of the remission and lead to repeated hospitalizations negatively influencing professional activity [19, 20]. This fact is especially important for individuals, who work in the conditions of constant psycho-emotional stress, and psycho-traumatic (stressful) events including railroad workers (train operators, assistants of train operators, traffic controller) [21, 22].

Pharmacological correction of combined pathology containing neurotic disorders is sufficiently complicated, especially in individuals working as operators [23]. Pharmaceutical effect on the professional abilities of train drivers is considered to be the issue of the day all over the world [24]. More than ten countries (Germany, France, Spain and others) have lists of drug products with indication of their danger level that is specified depending on their effect on the central nervous system when being taken by vehicle operators [25].

“Classification of drug products depending on the level of their negative effects on the professionally significant functions of train operators and other operators’ professions” developed by Tsfasman A.Z. et al. [23] in 2011 is currently a must in the Russian Federation when administering drug therapy for railroad workers.

Thus, timely diagnostics, effective and safe MADD and chronic pancreatitis pathogenetic therapy are very important for the treatment of railroad workers.

**Materials and methods.** The research study was made on the basis of the Clinical Pharmacology Department of the Voronezh N.N. Burdenko Medical University and Gastroenterology Department of the Non-state Health Care Facility “Road Hospital the Station Voronezh-1of JSC “Russian Railways”.

110 railroad workers admitted to the in-patient department with the diagnosis “Chronic pancreatitis, recurrent” (section K86.1 “Other chronic pancreatitis” of the International Classification of Diseases, 10th edition) were examine in the study. Among them there were 78 males and 32 females, average age 44.5±2.8, including 42 assistants of the train operators (38.2%), 29 train operators (26.4%), 20 train hosts (18.2%) and 19 train traffic controllers (17.2%); 24 healthy railroad workers, average age 42.4±1.7, formed a separate study group.

On admission all patients were questioned using specialized diagnostic scales and questionnaires aimed at revealing anxiodepressive disorders. These questionnaires included Hospital Anxiety and Depression Scale HADS (anxiety/ depression), Zung Anxiety Rating Scale ZARS (self-evaluation of depression), Spielberger-Khanin test (personal and reactive anxiety), a questionnaire “Health, Activity, Mood” (CAH in Russian). The diagnose “Mixed anxiety and depressive disorder” was made according to the International Classification of Diseases, 10th edition (section F41.2 “Mixed anxiety and depressive disorder”).

Modified graduated Visual Analogue Scale (VAS) was used to evaluate intensity of pain syndrome and gastroenterological symptoms; intensity of pain syndrome and dyspeptic disorders (nausea, bitter taste in the mouth, diarrhea) were assessed according to a 5-point system.

Activity of the inflammatory process of the pancreas was defined on the level of serum amylase, pancreatic amylase and lipase in the biochemical blood assay. Ultrasound examination of the pancreas was performed to specify its sizes, boundaries, structure and parenchymal echo-genicity.

Assessment of psycho-physiological functional status was performed using psycho-diagnostic complex “Select-M” («Селект-М» in Russian). Participants were tested in an isolated, sound proof,
darkened room. Intensity and lability of nerve processes were investigated by defining a simple motor response (m/sec) to the red signal, complex motor responses to green light and to red light taking into account decision-making time. To differentiate psycho-motor or motor inhibition critical frequency of fusion flicker (CFFF) test was performed. The reaction to a moving object including an average passing time value, an average response time out value was used to estimate balance between excitation and inhibition processes in the cerebral cortex. Study results were interpreted according to “Methodological instructions on performing psycho-physiological examinations in the railway locomotive facilities” (№ 310y, Ministry of Railways of the Russian Federation, December 1, 1999) [26].

Pharmacoeconomic analysis was performed using software application “Calculation of the individual treatment cost” (certificate of registration №2011610459, January 11, 2012) [27].

Statistical data were processed using software application “SPSS 9.0”; the mean value (X) of standard deviation was estimated by calculating a mean error of the arithmetical mean value (m). Non-parametric Wilcoxon signed-rank test was used to estimate confidence rating. Correlation dependence was determined according to Spearman method.

Results. Comparative analysis of two groups of patients was performed at the first stage of study to assess MADD influence on the course of chronic pancreatitis and psycho-physiological functions: patients with chronic pancreatitis (n=45) and patients suffering from chronic pancreatitis and MADD (n=65). The control group consisted of healthy railway workers (n=24); the rate of visual-motor reactions was the only parameter determined in this group.

The analysis of the results according to psycho-diagnostic scales giving an opportunity to diagnose anxious-depressive disorders has demonstrated that anxiety and depression level in patients with chronic pancreatitis and MADD on HADS (anxiety/ depression) and Zung (depression) scales exceeds similar findings in the group of patients with chronic pancreatitis without affective disorders in 1.9 – 2.6 times (p< 0.01), and normative values in 1.3 – 2 times (Table 1).

**Table 1.**

<table>
<thead>
<tr>
<th>Findings</th>
<th>Normative values</th>
<th>Patients with chronic pancreatitis (n=45)</th>
<th>Patients with chronic pancreatitis and MADD (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HADS, anxiety, points</td>
<td>≤7</td>
<td>5,4±1,2</td>
<td>13,9±1,7**</td>
</tr>
<tr>
<td>HADS, depression, points</td>
<td>≤7</td>
<td>3,8±0,9</td>
<td>9,2±2,1**</td>
</tr>
<tr>
<td>Zung Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression, points</td>
<td>&lt;50</td>
<td>34,1±1,7</td>
<td>63,9±2,1**</td>
</tr>
<tr>
<td>Spielberger – Khanin test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal anxiety, points</td>
<td>≤30</td>
<td>41,5±1,2</td>
<td>58,3±2,9**</td>
</tr>
<tr>
<td>Reactive anxiety, points</td>
<td>≤30</td>
<td>30,5±2,6</td>
<td>59,1±2,2**</td>
</tr>
<tr>
<td>Health, Activity, Mood test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health, points</td>
<td>≥5</td>
<td>4,2±0,3</td>
<td>2,2±0,5**</td>
</tr>
<tr>
<td>Activity, points</td>
<td>≥5</td>
<td>5,3±0,2</td>
<td>3,3±0,7**</td>
</tr>
<tr>
<td>Mood, points</td>
<td>≥5</td>
<td>4,4±0,7</td>
<td>3,1±0,3*</td>
</tr>
</tbody>
</table>

Note: * - p<0.01 – confidence rating with the results of patients suffering from chronic pancreatitis.

Study of psycho-emotional sphere according to Spielberger-Khanin test has revealed an increased level of reactive and personal anxiety in 29 patients (64.4%) with chronic pancreatitis and in all patients (100%) with combined chronic pancreatitis and MADD pathalogy, maximally expressed in patients with co-morbid pathology. Alongside with the high level of anxiety a significant decrease of findings on the “Health, Activity, Mood” test was recorded in patients suffering from affective disorders; this fact gives evidence of negative self-evaluation (Table 1).

Concerning somatic disorders all the examined patients complained of pain syndrome and dyspeptic disorders (nausea, bitter taste in the mouth, diarrhea). However, intensity of gastroenterological symptoms was different in the comparable groups. In patients with chronic pancreatitis having no any signs of anxiety and depression pain according to VAS Scale was moderate averaging 2.8±0.1 points (“a symptom is slightly revealed”, “a symptom is moderately revealed”), whereas patients with chronic pancreatitis and MADD evaluated intensity of stomach-ache of
psychogenic nature as 4.5±0.6 points ("a symptom is severely revealed", "a symptom is very severely revealed"). Findings “nausea, bitter taste in the mouth, altered defecation pattern" in patients with combined pathology exceeded similar findings in patients suffering only from chronic pancreatitis in 1.6 – 2.1 times (Table 2).

Table 2.

Gastroenterological findings according to VAS Scale (X±m).

<table>
<thead>
<tr>
<th>Findings</th>
<th>Patients with chronic pancreatitis (n=45)</th>
<th>Patients with chronic pancreatitis+MADD (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain syndrome, points</td>
<td>2.8±0.1</td>
<td>4.5±0.6*</td>
</tr>
<tr>
<td>Nausea, points</td>
<td>2.3±0.2</td>
<td>4.9±0.8**</td>
</tr>
<tr>
<td>Bitter taste in the mouth, points</td>
<td>2.1±0.5</td>
<td>3.6 ±0.3*</td>
</tr>
<tr>
<td>Altered defecation pattern (diarrhea), points</td>
<td>1.6±0.1</td>
<td>3.5±0.2**</td>
</tr>
</tbody>
</table>

Note: * - p<0.05, ** - p<0.01 – confidence rating with the results of patients suffering from chronic pancreatitis.

According to the results of biochemical blood assay estimating enzymatic status changes of serum amylase level, pancreatic amylase level and blood lipase were registered in 22 patients (33.8%) with chronic pancreatitis and MADD. Thus, the value of serum amylase was 124.5±2.9 U/l, level of pancreatic amylase achieved upper limit of normal being less than 54.4±3.2 U/l, blood lipase averaged 67.3±1.9 U/l. The results obtained exceeded normal values in 1.2 times.

In contrast to patients with neurotic disorders, elevated enzyme level was revealed in 34 testees (75.5%) of the group of patients with chronic pancreatitis and without MADD; that was by 55.2% more often comparing to patients with MADD. Serum amylase level amounted 206.7±4.7 U/l, pancreatic amylase level amounted 93.2±3.3 U/l, blood lipase level amounted 94.7±2.8 U/l; this exceeded normal values in 1.6 – 2 times and similar values in the group of patients with combined chronic pancreatitis and MADD in 1.4 – 1.7 times (p<0.05; p<0.05; p<0.01).

According to ultrasound (US) examination of the pancreas diffuse changes of the organ tissue were revealed in all patients: irregular indistinct boundaries, elevation of echogenicity, inhomogeneity of parenchyma. However, increased sizes of a gland were observed in 73.3% of patients with chronic pancreatitis and in 20% of patients with chronic pancreatitis and MADD only.

The obtained clinico-laboratory and instrumental data in the group of patients with chronic pancreatitis gave explanation to presence of gastroenterological complaints and supported principal diagnosis; whereas in the group of patients with combined pathology clinical findings to a greater extent indicated at presence of psycho-somatic disorders.

Evaluation of psycho-physiological functions in patients with chronic pancreatitis and without MADD demonstrated average values of visual-motor reactions rate; this fact indicated at the stability of processes of psycho-motor reactions and was comparable to the test performance rate in the group of healthy railroad workers (Table 3).

Table 3.

Findings of psycho-physiological functions prior to initiating therapy(X±m).

<table>
<thead>
<tr>
<th>Findings</th>
<th>Control group (n=24)</th>
<th>Patients with chronic pancreatitis (n=45)</th>
<th>Patients with chronic pancreatitis+MADD (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple motor response, m/sec</td>
<td>351.3±18.9</td>
<td>350.8±12.3</td>
<td>510.3±10.4*</td>
</tr>
<tr>
<td>CFFF, Hz</td>
<td>37.8±4.3</td>
<td>34.5±5.9</td>
<td>35.6±4.7</td>
</tr>
<tr>
<td>Complex motor response to red light, m/sec</td>
<td>419±16.8</td>
<td>508.2±10.6</td>
<td>626.9±8.8*</td>
</tr>
<tr>
<td>Complex motor response to green light, m/sec</td>
<td>461±21.6</td>
<td>452.5±19.5</td>
<td>685±17.1**</td>
</tr>
<tr>
<td>Decision-making time_red</td>
<td>115±22.8</td>
<td>135.6±19.6</td>
<td>179.5±18.3*</td>
</tr>
<tr>
<td>Decision making time_green</td>
<td>95.7±19.9</td>
<td>131.9±21.5</td>
<td>156.3±14.3</td>
</tr>
<tr>
<td>Reaction to a moving object including an average passing time value, m/sec</td>
<td>46.9±4.5</td>
<td>48.9±5.7</td>
<td>47.2±8.3</td>
</tr>
<tr>
<td>Reaction to a moving object including an average response time out value, m/sec</td>
<td>48.3±4.4</td>
<td>58.9±5.2</td>
<td>116.3±11.4**</td>
</tr>
</tbody>
</table>

Note: * - p<0.05, ** - p<0.01 – confidence rating with the results of patients suffering from chronic pancreatitis.

In the group of patients with co-morbid pathology aggravation of test results with manifestations of inhibiting reaction was registered in 78.4% of cases. Investigation of simple motor response demonstrated increased time of task performance by 45.5% (p<0.05) comparing to the result of test performance rate in the group of patients without anxiety and depression. The amount of CFFF


at that was 35.6±4.7 Hz, which is considered to be an average value and against the background of low simple motor response values is interpreted as the decrease of psycho-motor reaction. Test performance time in case of complex motor response to red and green light, as well as decision-making time to red light was respectively by 23.3%, 51.4% and 32.3% low than in patients with chronic pancreatitis (p<0.05, p<0.01; p<0.05). When performing the reaction to a moving object test in patients with chronic pancreatitis and MADD, the response time out reaction to a presented stimulus was prevalent. Average response time out values of the reaction to a moving object were 2 times higher in the group of patients without MADD (p<0.01) (Table 3).

The following observation groups were formed out at the second stage of research study considering character of the conducted therapy: the control group (n=45) – patients with chronic pancreatitis (principal disease) without signs of anxiety and depression receiving standard (basic) pharmacotherapy (enzymatic preparations, proton pump inhibitors, spasmyloytic drugs, non-narcotic analgetics, antidiarrheal preparations); the 1st group (n=25) – patients with combined chronic pancreatitis and MADD receiving only basic therapy against their principal disease; the 2nd group (n=20) patients with combined chronic pancreatitis and MADD receiving tranquilizer of nonbenzodiazepine series – Tetramethyltetraazabicyclooctandione (“Adaptol”) in addition to basic preparations; the 3rd group of patients taking “Afobazol” anxiety level also reduced from 13.9±2,1 to 10,2±0,6 points (p<0.05) on the 7th day of the therapy, to 9,3±1,2 points (p<0.01) in two weeks after administering the preparation, to 7,9±0,8 points (p<0.01) on the 30th day of the therapy (Figure 1).

In contrast to patients, who were given pharmacological correction of MADD, anxiety level in the 1st group of patients receiving only basic preparations was not significantly altered: 14±2,2 points before treatment, 13,3±2,6  points on the 7th day of the therapy, 13,6±3,1 points on the 14th day, 13,8±1,4 points on the 30th day; this exceeded upper normal limits in 1.9 times (Figure 1).

After being discharged from the hospital all testees continued taking enzymatic preparations and proton pump inhibitors out-patiently for 14 days. Psychotropic therapy was being performed up to 30 days.

The effectiveness and safety of treatment was evaluated on the 7th, 14th and 30th days of the treatment onset. Unfavourable side effects were being monitored during the whole period of observation for patients.

Significant decrease of the anxiety component was registered according to the HADS Scale against the background of psychotropic drugs administration. Anxiety value in the 2nd group of patients taking “Adaptol” decreased from 13.6±1,8 to 10,8±0,7 points (p<0.05) on the 7th day of the therapy, to 8,9±0,4 points (p<0.05) on the 14th day of the therapy, to 7,8±0,8 points (p<0.01) on the 30th day of examination. In the 3rd group of patients taking “Afobazol” anxiety level also reduced from 13.9±2,1 to 10,2±0,6 points (p<0.05) on the 7th day of the therapy, to 9,3±1,2 points (p<0.01) in two weeks after administering the preparation, to 7,9±0,8 points (p<0.01) on the 30th day of the therapy (Figure 1).

In contrast to patients, who were given pharmacological correction of MADD, anxiety level in the 1st group of patients receiving only basic preparations was not significantly altered: 14±2,2 points before treatment, 13,3±2,6  points on the 7th day of the therapy, 13,6±3,1 points on the 14th day, 13,8±1,4 points on the 30th day; this exceeded upper normal limits in 1.9 times (Figure 1).

Figure 1. Dynamics of the anxiety level according to the HADS Scale against the background of the complex therapy.

Note: * - p<0.05, ** - p<0.01- confidence rating with the results of examination before the treatment onset.
Control of the depression level according to the HADS and Zung Scales on the 7th and 14th day of the therapy did not reveal significant dynamics of the indicated value in both - patients receiving anxiolytics (“Adaptol”, “Afobazol”) and patients, who were administered only basic therapy of chronic pancreatitis. The depression value according to HADS and Zung Scales in the 1st group of patients was at the same level on the 30th day of examination, whereas it had a statistically non-significant tendency to reduce in the 2nd and 3rd groups.

According to Spielberger-Khanin test the reactive anxiety value in the control group was 28.6±2.2 points against the background of the basic therapy on the 14th day of treatment; the obtained values were the same on the 30th day of examination. Personal anxiety level had no changes during the whole course of the therapy being 37.5±2.1 points on the 7th day of treatment, 42.6±2.8 points on the 14th day and 38.6±3.2 points on the 30th day. Results demonstrated low level of reactive anxiety and moderate level of personal anxiety (Table 4).

<table>
<thead>
<tr>
<th>Value</th>
<th>Before treatment</th>
<th>7th day of treatment</th>
<th>14th day of treatment</th>
<th>30th day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal anxiety, points</td>
<td>41.5±1.2</td>
<td>37.5±2.1</td>
<td>42.6±2.8</td>
<td>38.6±3.2</td>
</tr>
<tr>
<td>Reactive anxiety, points</td>
<td>30.5±2.6</td>
<td>32.6±3.5</td>
<td>28.6±2.2</td>
<td>28.3±2.9</td>
</tr>
<tr>
<td><strong>Control group (n=45)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal anxiety, points</td>
<td>61.3±2.1</td>
<td>58.7±1.8</td>
<td>53.5±1.9</td>
<td>65.8±3.6</td>
</tr>
<tr>
<td>Reactive anxiety, points</td>
<td>56.7±0.9</td>
<td>52.3±0.5</td>
<td>53.6±1.2</td>
<td>54.2±2.8</td>
</tr>
<tr>
<td><strong>1st group (n=25)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal anxiety, points</td>
<td>59.7±1.9</td>
<td>55.2±2.4</td>
<td>44.1±2.2*</td>
<td>45.6±1.9*</td>
</tr>
<tr>
<td>Reactive anxiety, points</td>
<td>60.2±1.2</td>
<td>54.3±2.4</td>
<td>48.3±1.6*</td>
<td>42.6±1.6*</td>
</tr>
<tr>
<td><strong>2nd group (n=20)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal anxiety, points</td>
<td>63.2±2.2</td>
<td>58.2±1.7</td>
<td>48.7±2.4*</td>
<td>47.8±2.1*</td>
</tr>
<tr>
<td>Reactive anxiety, points</td>
<td>56.5±1.2</td>
<td>49.7±2.7</td>
<td>37.4±4.2**</td>
<td>32.4±3.5**</td>
</tr>
<tr>
<td><strong>3rd group (n=20)</strong></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

Note: * - p<0.05, ** - p<0.01 - confidence rating with the results of examination before the treatment onset.

In the 1st group of patients with co-morbid pathology, who were given therapy only for chronic pancreatitis, values of personal and reactive anxiety during the whole period of in-patients and out-patients treatment were at the same level as before treatment being more than 46 points (high level of anxiety).

A significant decrease of anxiety values was registered on the 14th day of the therapy against the background of “Adaptol” and “Afobazol” administration. On the 30th day of examination personal and reactive anxiety values in patients of the 2nd group receiving “Adaptol” were at the obtained level being less than 46 points (moderate anxiety). In contrast to the 2nd group of patients intensity of reactive anxiety significantly reduced in patients of the 3rd group taking “Afobazol” – by 42.6% comparing to the initial level and obtained 32.4±3.5 points (< 30 points is considered to be low level of anxiety).

According to the results of “Health, Activity, Mood” test health and mood findings in patients of the control group tended to increase amounting to 5.8±0.2 points (p<0.05) and 5.4±0.2 points respectively (p<0.05) on the 14th day of treatment. Activity findings were at the sufficiently high level of 5.5±0.2 points as before treatment. On the 30th day of examination health, activity, mood findings were within normal limits (more than 5 points) (Table 5).

In the 1st group of patients with chronic pancreatitis and MADD, who were given only standard pharmacotherapy, there were no significant improvements on the 7th, 14th and 30th days according to “Health, Activity, Mood” test. Health, activity and mood values in patients were at the same low level as before treatment being no more than 3.4 ±0.5 points.

Positive self-evaluative dynamics of the patients’ condition according to “Health, Activity, Mood” test was recorded in the 2nd and 3rd group of patients with combined pathology taking tranquilizers alongside with basic preparations in 2 weeks of pharmacotherapy. Thus, health and mood values increased by 78.8% and 30.5% respectively (p<0.01, p<0.05) on the 14th day of treatment obtaining 5 and more points score on the 30th day of treatment. Activity level at that was low during the whole period of study. Opposed to “Adaptol”, “Afobazol” had a positive impact not only on the health and mood, but also on the activity level. All findings tended to increase by 73.9%, 37.5% and 53.3% respectively (p<0.05, p<0.01) on the 14th day of treatment obtaining more than 5 points score on the 30th day of examination.
Results of psychological test according to “Health, Activity, Mood” questionnaire against the background of pharmacotherapy (X±m).

<table>
<thead>
<tr>
<th>Value</th>
<th>Before treatment</th>
<th>7th day of the therapy</th>
<th>14th day of the therapy</th>
<th>30th day of the therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=45)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health, points</td>
<td>4,2±0,3</td>
<td>4,8±0,1</td>
<td>5,8±0,2*</td>
<td>5,6±0,2</td>
</tr>
<tr>
<td>Activity, points</td>
<td>5,3±0,2</td>
<td>5,2±0,5</td>
<td>5,5±0,2</td>
<td>5,3±0,3</td>
</tr>
<tr>
<td>Mood, points</td>
<td>4,4±0,7</td>
<td>4,6±0,2</td>
<td>5,4±0,4*</td>
<td>5,4±0,2**</td>
</tr>
<tr>
<td>1st group (n=25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health, points</td>
<td>2,5±0,7</td>
<td>2,9±0,3</td>
<td>3,2±0,6</td>
<td>2,7±0,3</td>
</tr>
<tr>
<td>Activity, points</td>
<td>3,5±0,3</td>
<td>3,1±0,5</td>
<td>3,4±0,5</td>
<td>2,9±0,2</td>
</tr>
<tr>
<td>Mood, points</td>
<td>2,9±0,1</td>
<td>2,2±0,2</td>
<td>2,5±0,2</td>
<td>3,4±0,2</td>
</tr>
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<td>2nd group (n=20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health, points</td>
<td>1,9±0,5</td>
<td>2,8±0,3</td>
<td>3,4±0,2,2**</td>
<td>5±0,3**</td>
</tr>
<tr>
<td>Activity, points</td>
<td>3,1±0,3</td>
<td>3,3±0,2</td>
<td>2,9±0,4</td>
<td>3,7±0,5</td>
</tr>
<tr>
<td>Mood, points</td>
<td>3,6±0,2</td>
<td>4,2±0,2</td>
<td>4,7±0,4*</td>
<td>5,2±0,3**</td>
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<tr>
<td>3rd group (n=20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health, points</td>
<td>2,3±0,5</td>
<td>3,9±0,2</td>
<td>4±0,2**</td>
<td>5,4±0,6**</td>
</tr>
<tr>
<td>Activity, points</td>
<td>3,2±0,2</td>
<td>3,5±0,5</td>
<td>4,4±0,3**</td>
<td>5,2±0,2**</td>
</tr>
<tr>
<td>Mood, points</td>
<td>3±0,2</td>
<td>4,2±0,1</td>
<td>4,6±0,2*</td>
<td>5,2±0,2**</td>
</tr>
</tbody>
</table>

Note: * - p<0.05, ** - p<0.01 - confidence rating with the results of examination before the treatment onset.

According to VAS reduction of pain syndrome from 2,8±0,1 points to 1,6±0,1 points (p<0,01) was registered in the group of patients with chronic pancreatitis and without MADD on the 7th day of pharmacotherapy with the followed positive dynamics on the 14th day of therapy when pain intensity in 86.8% of patients amounted to 1 point (p<0,01); that indicated at the “lack of symptom”. On further examination 93.3% of patients reported the pain intensity being equal to 0 point (p<0,01) when completing VAS questionnaire in 30 days after the treatment onset. On the contrary, no positive dynamics was observed on the value “stomach-ache of psycho-genic nature” in patients of the 1st group with combined pathology on the 7th, 14th and 30th days of the therapy. In the 2nd group of patients taking “Adaptol” pain intensity level decreased from 3,9±0,2 to 2,5±0,6 points (p<0,05) on the 14th day and to 1,8±0,2 points (p<0,01) on the 30th day of the therapy. In the 3rd group of patients receiving “Afobazol” a significant decrease of pain syndrome from 4,3±0,1 to 2,7±0,8 points (p<0,05) was registered as early as on the 7th day of the therapy with the followed tendency to reduce up to 1,5±0,1 points (p<0,01) on the 14th day of examination; that was comparable with the results of treatment in patients of the control group with chronic pancreatitis. On the 30th day of examination the value “stomach-ache of psycho-genic nature” remained at the obtained level - 1,5±0,2 points (p<0,01) (Figure 2).

Evaluation of dynamics of other gastrointestinal symptoms in patients of the control group revealed gradual decrease of nausea intensity, bitter taste in the mouth and diarrhea. On the 7th day of treatment nausea intensity decreased from 2,3±0,2 to 1,9±0,6 points (p<0,05), bitter taste in the mouth – from 2,1±0,5 points to 1,7±0,4 and diarrhea – from 1,6±0,1 points to 1,3±0,3 points. On the 14th day of treatment of chronic pancreatitis exacerbation the above-mentioned values in 87.7% of patients did not exceed 1 point score (p<0,01; p<0,01; p<0,05), that indicated at the lack of gastrointestinal manifestations. On the 30th day of the therapy no patients complained of gastrointestinal disorders.

Patients of the 1st group reported about dyspeptic disorders at the same level as before the treatment onset comparing to patients of the control group. During the whole course of treatment severity of nausea, bitter taste in the mouth and diarrhea were equal to 3 – 4 points; this indicated at the “moderately revealed” and “severely revealed” symptoms. Diarrhea status decreased by 23.7% (p<0,05) on the 30th day of the therapyonly.

When performing complex treatment with application of psychotropic preparation severity of dyspeptic disorders also tended to decrease. In patients of the 2nd group, who received “Adaptol” alongside with the basic therapy, somatic findings improved on the 14th day of treatment – nausea severity decreased from 4,7±0,2 to 2,5±0,9 points (p<0,01), diarrhea severity decreased from 3,9±0,2 to...
2.4±0.5 points (p<0.05); complaints on the bitter taste in the mouth remained unchanged, its intensity level amounted 3.2±0.9 points as before treatment. On the 30th day of treatment severity level of nausea and diarrhea amounted 1-2 points (p<0.01), this indicated at the “slightly revealed symptom”. Severity of nausea and diarrhea at that amounted 1 point (“lack of symptom”) in 45% of patients. Severity of the bitter taste in the mouth was without essential dynamics - 2.9±0.4 points. In contrast to “Adaptol” intake “Afobazol” administration allowed receiving therapeutical results as early as the 7th day of treatment; this was manifested in significant decrease of complaints on nausea from 4.6±0.2 to 3.4±1.2 points (p<0.05), bitter taste in the mouth - from 4.05±0.7 to 3.2±0.3 points (p<0.05); severity of diarrhea tended to decrease from 3.8±0.2 to 3.2±0.3 points. On the 14th day of treatment the above-mentioned symptoms did not exceed 2.6±0.2 points; on the 30th day of examination 55% of patients did not complain of nausea, bitter taste in the mouth and diarrhea (Figure 2).

Control of biochemical findings against the background of the performed treatment demonstrated favourable effect of basic and complex therapy on the enzymatic status; this was manifested by the significant decrease of serum amylase, pancreatic amylase and blood lipase level.

Analysis of the rate of visual-motor reactions in the group of patients with chronic pancreatitis having no MADD (the control group), who received basic preparations, showed that there were no negative changes in the rate of visual-motor reactions; this indicated at the safety administration of preparations applied for the treatment of chronic pancreatitis in the context of professional operational activity.

The rate of visual-motor reactions in patients with chronic pancreatitis and MADD, who received
only basic preparations (the 1st group) was not altered during the whole course of in-patient and out-patient treatment. Rate of a simple motor response, a complex motor response, a reaction to a moving object was low with prevailing processes of inhibition and decrease of psycho-motor reaction.

In the 2nd group of patients, who were additionally administered “Adaptol”, test performance time of a simple motor response increased by 32% comparing to the initial level (p<0.05) on the 14th day of treatment; decision-making time (to green light) tended to increase by 27% comparing to the given parameter on the 14th day of examination (p>0.05). The results obtained were interpreted as the tendency to slow the rate of visual-motor reactions (Table 6).

Findings of psycho-physiological functions in patients with chronic pancreatitis and MADD against the background of complex therapy including “Adaptol”(X±m).

<table>
<thead>
<tr>
<th>Findings</th>
<th>Before treatment</th>
<th>7th day of treatment</th>
<th>14th day of treatment</th>
<th>30th day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple motor response, m/sec</td>
<td>465,2±8,7</td>
<td>478,3±12,9</td>
<td>615,7±11,5*</td>
<td>614,2±12,9*</td>
</tr>
<tr>
<td>CFFF, Hz</td>
<td>33,6±2,5</td>
<td>38,9±4,7</td>
<td>36,6±7,2</td>
<td>34,3±2,9</td>
</tr>
<tr>
<td>Complex motor response to green light, m/sec</td>
<td>698,1±11,4</td>
<td>697,5±15,8</td>
<td>679,3±13,9</td>
<td>665,4±16,7</td>
</tr>
<tr>
<td>Complex motor response to green light, m/sec</td>
<td>701,5±17,3</td>
<td>679,5±12,1</td>
<td>686,4±10,4</td>
<td>698,7±11,8</td>
</tr>
<tr>
<td>Decision-making time&lt;sub&gt;green&lt;/sub&gt;</td>
<td>156,4±15,6</td>
<td>147,2±15,6</td>
<td>158,3±12,5</td>
<td>198,7±16,8*</td>
</tr>
<tr>
<td>Decision-making time&lt;sub&gt;red&lt;/sub&gt;</td>
<td>176,4±12,4</td>
<td>167,3±18,2</td>
<td>166,9±22,6</td>
<td>169,7±15,8</td>
</tr>
<tr>
<td>Average passing time, m/sec</td>
<td>50,1±4,9</td>
<td>44,6±5,2</td>
<td>58,7±7,2</td>
<td>47,3±5,7</td>
</tr>
<tr>
<td>Average response time out value, m/sec</td>
<td>110,2±9,6</td>
<td>114,3±8,3</td>
<td>117,3±4,6</td>
<td>112,6±6,6</td>
</tr>
</tbody>
</table>

Note: * - p<0.05 - confidence rating with the results of examination before the treatment onset; # -p<0.05  p<0.05 - confidence rating with the results of examination on the 14th day of treatment.

In contrast to “Adaptol” intake administration of “Afobazol” reduced task performance time: performance time of a simple motor response decreased by 22.8% on the 7th day of therapy (p<0.05); performance time of a complex motor response to green light decreased by 30.2% (p<0.05), to red light – by 25.4% (p<0.05) on the 14th day of treatment; a reaction to a moving object including average response time out value decreased by 19.5%, a reaction to a moving object including average passing time value remained unchanged. In 2 weeks of anxiolytic “Afobazol” intake psycho-physiological findings reached average values of reaction to the presented stimulus typical for patients without MADD. On the 30th day of treatment performance time of a simple motor response, CFFF, a complex motor response, a reaction to a moving object reached normative values conforming with the average reaction time to a signal (Table 7).

Findings of psycho-physiological functions in patients with chronic pancreatitis and MADD against the background of complex therapy including “Afobazol”(X±m).

<table>
<thead>
<tr>
<th>Findings</th>
<th>Before treatment</th>
<th>7th day of treatment</th>
<th>14th day of treatment</th>
<th>30th day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple motor response, m/sec</td>
<td>472,5±12,9</td>
<td>364,3±9,2*</td>
<td>325,8±8,9</td>
<td>337,7±10,3</td>
</tr>
<tr>
<td>CFFF, Hz</td>
<td>38,8±2,9</td>
<td>40,6±4,8</td>
<td>39,7±6,9</td>
<td>37,4±7,5</td>
</tr>
<tr>
<td>Complex motor response to green light, m/sec</td>
<td>718,2±12,2</td>
<td>686,9±13,9</td>
<td>501,5±7,7*</td>
<td>513,7±8,9*</td>
</tr>
<tr>
<td>Complex motor response to green light, m/sec</td>
<td>689,2±10,2</td>
<td>702,3±8,4</td>
<td>513,9±7,2*</td>
<td>527,5±9,8*</td>
</tr>
<tr>
<td>Decision-making time&lt;sub&gt;green&lt;/sub&gt;</td>
<td>158,3±13,6</td>
<td>164,6±6,6</td>
<td>139,2±15,4</td>
<td>146,7±13,9</td>
</tr>
<tr>
<td>Decision-making time&lt;sub&gt;red&lt;/sub&gt;</td>
<td>184,7±15,8</td>
<td>179,5±14,7</td>
<td>149,2±15,4</td>
<td>156,7±13,9</td>
</tr>
<tr>
<td>Average passing time, m/sec</td>
<td>44,2±8,3</td>
<td>45,6±7,3</td>
<td>51,7±3,9</td>
<td>43,8±5,9</td>
</tr>
<tr>
<td>Average response time out value, m/sec</td>
<td>114,6±5,9</td>
<td>97,2±8,8</td>
<td>92,3±5,9*</td>
<td>94,3±7,8*</td>
</tr>
</tbody>
</table>

Note: * - p<0.05, ** - p<0.01- confidence rating with the results of examination before the treatment onset.
At the third stage of study pharmaco-economic analysis was performed. For this all the patients were divided into 4 groups: the 1\textsuperscript{st} group (n=45) – patients with chronic pancreatitis, who received standard therapy for the principal disease; the 2\textsuperscript{nd} group (n=25) – patients with chronic pancreatitis and MADD, who were administered only basic preparation; the 3\textsuperscript{rd} group (n=20) – patients with chronic pancreatitis and MADD, who received anxiolytic “Adaptol” alongside with the standard treatment; the 4\textsuperscript{th} group (n=20) – patients with chronic pancreatitis and MADD, who were administered basic preparations and anxiolytic “Afobazol”.

Performed pharmaco-economic analysis showed that treatment cost with gastroenterological preparations in the 2\textsuperscript{nd} group of patients with the combine pathology, who were not administered MADD correction, exceeded treatment cost of patients without affective pathology in 2 times (p<0.01) and in the group of patients receiving psychotropic medications “Adaptol” or “Afobazol” in 1.6 and 1.5 times respectively (p<0.05) (cost of these medications was not taken into account) (Figure 3).

Administration of psychotropic therapy to patients with MADD reduced spasmyotics intake by 24.1\%, analgesics intake by 23.9\% and prokinetics intake by 28.5\% in average, due to the effective elimination of the pain syndrome and dyspeptic disorders, since their intensity, as it was shown in the study, was related to the affective disorders. As a result, the therapy course with indicated preparations in patients with chronic pancreatitis and MADD receiving psycho-pharmacotherapy was practically approaching to the course dose of preparation intake in patients with chronic pancreatitis without signs of anxiety and depression, and therapeutical costs were consequently lower (Table 8).

### Table 8

<table>
<thead>
<tr>
<th>Group</th>
<th>Spasmyotics, days</th>
<th>Analgesics, days</th>
<th>Prokinetics, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{st} group (n=45)</td>
<td>7.2±0.2</td>
<td>5.3±0.2</td>
<td>5.4±0.3</td>
</tr>
<tr>
<td>2\textsuperscript{nd} group (n=25)</td>
<td>10.6±1.8</td>
<td>9.2±1.1</td>
<td>8.4±1.2</td>
</tr>
<tr>
<td>3\textsuperscript{rd} group (n=20)</td>
<td>7.9±1.3</td>
<td>7.2±0.4</td>
<td>5.8±1.2</td>
</tr>
<tr>
<td>4\textsuperscript{th} group (n=20)</td>
<td>8.2±1.4</td>
<td>6.8±1.5</td>
<td>6.2±1.4</td>
</tr>
</tbody>
</table>

Complex therapy cost estimation considering cost of psychotropic preparations showed that “Adaptol” administration increased chronic pancreatitis and MADD treatment costs by 58.9\%, whereas additional intake of “Afobazol” did not cause any significant price elevation, expenses at that rose by 26.6\%.

When evaluating an individual tolerability of administered psychotropic preparations, patients were interviewed to reveal adverse drug reaction (ADR)
typical for medications of tranquilizers group (sleepiness during the daytime, muscle relaxation and drug addiction), as well as side effects described in the instruction for medical use of “Adaptol” (dizziness, decrease of blood pressure, fatigue, dyspeptic disorders, allergic reactions) and “Afobazol” (allergic reactions, headache).

No unfavourable effects or apparent health deterioration demanding cancellation of preparation or daily dose decrease were registered during the observation against the background of “Adaptol” and “Afobazol” therapy.

When analyzing an individual tolerability of “Adaptol” 70% of patients complained of the apparent bitter taste in the mouth appearing right after the preparation intake and occurring during the whole therapy course. Besides, 55% of patients spoke about inconvenience of taking the preparation, which was related to the large size of a tablet. One patient noted intensification of dizziness during the first 10 days of “Adaptol” therapy. However, no significant changes of blood pressure and pulse rate were registered at the checkups.

10% of patients in the group of patients receiving “Afobazol” had sleeping disorders in the form of sleepiness during the daytime, which appeared on the 3-4 day of the treatment onset and compensated spontaneously on the 5-7 day of treatment. One patient, who was emotionally excited about his psycho-somatic status even before the psycho-pharmacoreaction therapy onset, revealed prevalence of depressive component in the psychological testing, his reactive and personal anxiety was of high level; after the treatment course he reported about the development of “withdrawal symptoms” and, as a result, drug addiction to “Afobazol”. 30% of patients did not agree with the frequent daily intake of the preparation (3 times a day), but they followed therapy regimen and fully completed it.

Discussion. Examination of the patients suffering from chronic pancreatitis demonstrated that patients with MADD had more severe course of the disease. This manifested by intensive pain syndrome, apparent symptoms of nausea, bitter taste in the mouth and diarrhea exceeding similar complaints in patients with chronic pancreatitis having no any sign of anxiety and depression in 1.6-2.1 times.

The fact that in 75.5% of patients with chronic pancreatitis without MADD biochemical findings (serum amylase, pancreatic amylase, blood lipase) exceeded normative values in 2 times and US examination of the pancreas revealed its enlargement was of great significance. In contrast to patients without signs of anxiety and depression elevated level of enzymes was registered only in 38.8% of patients with combined pathology exceeding upper normative limits in 1.2 times, and US examination results indicated at the alteration of gland tissue without altering its sizes. The obtained data conform to research studies performed earlier, which demonstrated that it is impossible to reveal objective and significant gastro-intestinal changes in somatic patients with neurotic disorders during thorough repeated laboratory and instrumental investigations [28].

Psychodiagnostic test results in patients with co-morbid pathology revealed prevalence of the anxiety level that was 2 times higher than normative values according to HADS and Zung Scales. Survey results demonstrated that more than half a number of all patients were under conditions of constant stress for a long period of time (3 – 7 years). They reported about severe labour environment, shift basis working hours including night shifts, feeling of excessive responsibility at their working place. This supports the fact of developing anxiodepressive disorders mostly in patients, whose professional activity is subjected to long-lasting psycho-emotional overstrain [29, 30].

According to test results 100% of patients with co-morbid pathology revealed elevated level of reactive and personal anxiety with the intensity of 58,3±2,9 and 59,1±2,2 points (46 points and more indicate at the high level of anxiety). The data obtained support the fact that elevation of situational and personal anxiety in locomotive crew workers increases risk of developing pathological somato-vegetative violations that cause inner organ disorders and deterioration of psychophysiological functions [31].

Assessment of combine pathology impact on the rate of visual-motor reactions appears to be an important aspect of study. The obtained results estimate presence of inhibiting reaction in 78.4% of patients. For example, rate of simple motor response was decreased in 45.5% comparing to the analogue reaction in patients without MADD (p<0.05). CFFF at that was in the range of average values and in the context of low simple motor response values was interpreted as decrease of psycho-motor reaction (according to “Methodological instructions on performing psycho-physiological examinations in the railway locomotive facilities” № 310y, Ministry of Railways of the Russian Federation, December 1, 1999). Test performance time for a complex motor response to green and red light was by 23.3% and 51.4% more than in patients with chronic pancreatitis without affective pathology (p<0.01, p<0.05). According to results of the reaction to a moving object test average response time out values exceeded normative values amounting 116.7±11.4 m/sec (p<0.01) with normative values of an average passing time – 47.2±8.3 m/sec.
As a psychotropic therapy preparations patients were administered non-benzodiazepine tranquilizer Tetramethyltetrazabicyclooctandione (“Adaptol”) in a daily dose 1000 mg and selective non-benzodiazepine anxiolytic Fabomotizole (“Afobazol”) in a daily dose 30 mg. Administration of the given preparations is pathogenically proved, since their action is aimed at the strengthening of GABAergic and serotoninergic mechanisms in the brain [32, 33].

Impact of preparations on the rate of visual-motor reactions revealed during the research study appeared to be a restriction to apply “Adaptol” in train operators, assistants of train operators, traffic controllers at the outpatient stage of treatment. Only train hosts continued receiving this preparation after being discharged from the hospital. In 2011 “Adaptol” was included in the list of the 1st class preparations that are considered to be dangerous for the on-the-job administration for train operators and their assistants, since it can result in hypertension, fatigue and dizziness (study guidelines, IVth edition, updated and revised, “Medications and safety of train traffic” by Professor Tsfasman et al., 2011). Changes indicating at the “Adaptol” potential to negatively influence the ability to drive transport vehicle and serve mechanisms causing possible decrease of blood pressure and fatigue were included in the Instruction for medical use of preparation (2011) [34].

Observation results of patients suffering from chronic pancreatitis and MADD demonstrate that clinical use of preparations of the non-benzodiazepine tranquilizer group “Adaptol” and “Afobazol” as a part of complex therapy allows conducting effective pharmacotherapy with favourable impact on gastrointestinal and anxiety symptoms.

In the context of “Adaptol” administration the level of pain syndrome decreased by 35.8% on the 14th day of the in-patient treatment alongside with the reduction of nausea by 46.8% and diarrhea by 38.4%. The indicated findings amounted 1 point score in 45% of patients on the 30th day of the out-patient treatment; that indicated at the “lack of symptom”, though bitter taste in the mouth remained in more than half a number of patients during the whole course of therapy.

“Afobazol”, in contrast to “Adaptol”, allowed obtaining therapeutical results as early as on the 7th day of treatment. Pain syndrome decreased by 37.2%, nausea – by 26% and bitter taste in the mouth – by 20.9%. In a month after the onset of the preparation administration 55% of patients did not complain of dyspeptic disorders.

In patients with chronic pancreatitis and MADD, who were not given psycho-pharmaco-correction, chronic pancreatitis treatment was ineffective. Patients were discharged with complaints on the apparent stomach-ache of psycho-genic nature, nausea and altered defecation pattern. Thus, MADD may provide developing of drug resistance and negative therapeutic outcome for patients with chronic pancreatitis.

Dynamics evaluation of anxi-depressive disorders in the context of administration of psychotropic drugs gave an opportunity to reveal a significant decrease of anxiety level on the 7th day of “Adaptol” and “Afobazol” administration; their values went down by 20.5% (p<0.05) and 28% (p<0.05) respectively. Depression level tended to decrease on the 30th day of therapy. A significant decline of reactive and personal anxiety was registered on the 14th day in the context of “Adaptol” and “Afobazol” administration, however, in 30 days of treatment “Afobazol” influence on situational anxiety was more evident and amounted 32.4±3.5 points, whereas “Adaptol” decreased reactive anxiety level up to 42.6±1.6 points only.

Research results aimed at the assessment of tranquilizers impact on the psycho-physiological functions in railroad workers demonstrated certain peculiarities. In the context of “Adaptol” administration the time of a simple motor response test performance increased by 32% comparing to the initial level (p<0.05), and decision-making time to green light tended to increase by 27% comparing to the same value on the 14th day of treatment. The obtained alterations support the fact that preparation has a potential to reduce rate of visual-motor reactions that restricts use of the given medication in locomotive crew workers.

“Afobazol”, on the contrary, reduced time of task performance. This manifested in the decrease of a simple motor response values by 22.8% (p<0.05), a complex motor response to green light values by 30.2% (p<0.05), to red light by 25.4% (p<0.05), a reaction to a moving object values by 19.5% (p<0.05). The obtained results have an important significance for railroad workers, whose professional activity demanded boosting attention span and reaction fast rate. Positive “Afobazol” impact on the rate of visual-motor reactions has become a reason for execution of a patent for invention (“Way of increasing psycho-motor reaction rate using “Afobazol” anxiolytic”, patent № 2528110, registration date 16.07.2014) [35]; conducted psycho-physiological research study gives an opportunity to determine an effective and safe preparation for
railroad workers when performing pharmacological correction of anxiodepressive disorders.

Based on pharmaco-economic analysis and considering high efficiency and low cost of the course treatment a therapy design including anxiolytic “Afobazol” is considered to have higher priority.

To conclude, it is necessary to report that results of the current study have practical significance for patients with combine pathology including chronic pancreatitis and anxiodepressive disorders, since conducted complex assessment of the gastrointestinal status, peculiarities of psycho-emotional sphere and psycho-physiological functions give an opportunity to determine pathogenic peculiarities of co-morbid diseases and choose the most rational scheme of pharmaco-correction.

Conclusions.

1. Combination of chronic pancreatitis and anxiodepressive disorders provide worsening of pain syndrome and gastro-intestinal disorders resulting in resistance to the conducted therapy.

2. Anxiodepressive syndrome, co-morbid with chronic pancreatitis, reduces rate of visual-motor reactions increasing performance time of a simple motor reaction by 45%, a complex motor reaction by 37% and a reaction to a moving object in 2 times comparing to similar findings of patients with chronic pancreatitis without anxiety disorders.

3. Complex therapy including administration of a tranquilizer “Adaptol” provides regress of anxiety on the 7th day of treatment, gastroenterological symptoms on the 14th day of treatment, whereas an “Afobazol” anxiolytic simultaneously eliminates both - anxiety and gastroenterology disorders, on the 7th day of treatment.

4. “Afobazol” anxiolytic administration as a part of complex therapy in case of exacerbation of chronic pancreatitis ensures elevated rate of visual-motor reactions in 80% of patients, whereas “Adaptol” tranquilizer affects psycho-physiological findings in 65% of cases.

5. Additional administration of an “Adaptol” tranquilizer increases treatment cost in 2.4 times, whereas application of “Afobazol” increases treatment cost in 1.4 times.

References


8. Kotova, O.V. Potential of psycho-vegetative syndrome therapy. Difficult Patient, 2011, № 12, pp. 31-34. (In Russian) [eLIBRARY] [Full text]


10. Garipova, Y.A. Various ways of pharmacocorrection of allied psychotic disorders in patients with chronic pancreatitis (Candidate of Medical Sciences diss., Ufa, 2011). (In Russian) [eLIBRARY] [Full text]

11. Shevchenko, Y.M. Diagnostics of non-psychotic psychic disorders in patients with chronic pancreatitis: GP experience. Family Medicine, 2015, №3 (59), pp. 61. (In Russian) [eLIBRARY] [Abstract]


13. Kharkina, D.N. Non-psychotic psychic disorders in patients with chronic pancreatitis and their correction (Candidate of Medical Sciences diss., Voronezh, 2007) (In Russian) [eLIBRARY] [Full text]


Gastroenterology, 2005, № 4, pp. 962-974. [PubMed] [Full text]


22. Chitlova, V.V. Anxious depression and personality disorders (Candidate of Medical Sciences diss., Moscow, 2013) (In Russian) [eLIBRARY] [Full text]

23. Tsfasman A.Z., Gutnikova O.V., Gorokhov S.G. et al. Medications and safety of train traffic (Moskva, 2011), 64 p. (In Russian) [eLIBRARY]


30. Nuzhdina, A.A. Peculiarities of psycho-emotional status and arterial hypertension course in mental workers. Occupational medicine and industrial ecology, 2008, № 4, pp. 8-12. (In Russian) [eLIBRARY] [Full text]


34. State register of drug products, (accessed date: December 22, 2016) http://www.grls.rosminzdrav.ru/ (In Russian) [Full text]

35. Lyubavskaya S.S., Chernov Y.N., Batisheva G.A., Goncharova N.Y. The way to increase of psychomotor reactions rate with anxiolytic Afobazol. Patent RF № 2528110, 2014. [eLIBRARY] [Full text]
The main criterion for the development of new drugs is their pharmacological efficacy, so the purpose of the research was the studying of the influence of citrates and malates of biogenic metals on physiological and biochemical parameters of the tissues and organs of broiler chickens in order to justify the optimal conditions for their use to stimulate the productivity of the birds.

The structure of the obtained agents was identified based on the content of the metal by atomic absorption spectroscopy, on the basis of data of infrared spectroscopy, data, H-element analysis.

The studied citrates of manganese, zinc, iron and cobalt are characterized by a strong absorption band at 1558 cm⁻¹ and 1560 cm⁻¹, which characterises the complex compounds with a chelate bond.

The assignment of frequencies to one or another group was carried out on the basis of the known literature data [7].

The data of IR-spectra shows that citrates of zinc, iron, manganese, and cobalt, obtained in the given technological conditions practically don’t contain free carboxyl groups, i.e., all three carboxyl groups are linked with the ion of the transition metal. The carbonyl groups of acidic residues and the tertiary hydroxyl are also associated diversely. Atom of the transition metal coordinates the donor atoms, where in the formed
complexes have a chelate (cyclic) structure due to coordination of metal ion simultaneously by several donor-active groups of citric acid (ionized carboxyl groups and hydroxyl group).

The major determining factors the composition and the proportion of the dominant forms in the products of the interaction of transition metal ions with citric acid are the pH of the solution at the final stage of synthesis and the ratio of the concentrations of the reacting substances.

Regardless of the composition of the initial samples (in solid state), its dissolution, including in the gastrointestinal tract of animals, causes the conversion of initial forms in the form which are the most stable at a pH characterising the acidity of the solution.

The high stability of the dominant forms of malate and citrate complexes of the studied metal ions should determine the prolonged effects of drugs on the basis of the study samples [8].

Material and methods.

The experiment was conducted using a total of 175 “Ross-308” broiler chickens at the age interval of 1 to 42 days. Broilers were randomly allotted into seven groups, 25 birds in each.

In the first control group chickens fed the inorganic compounds like iron sulphate, manganese carbonate and zinc oxide (FeSO4, Mn (CO3)2, ZnO) in the combination fodder. In the feed for the chicken of the second, third and fourth groups the inorganic compounds of the trancer elements were replaced by malate; in the fifth, sixth and seventh groups - by citrate metals. Doses are presented in table 1.

Table 1.

<table>
<thead>
<tr>
<th>Dose, g/ton</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Malates:</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>19</td>
</tr>
<tr>
<td>Mn</td>
<td>75</td>
</tr>
<tr>
<td>Zn</td>
<td>53</td>
</tr>
<tr>
<td>Citrates:</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>–</td>
</tr>
<tr>
<td>Mn</td>
<td>–</td>
</tr>
<tr>
<td>Zn</td>
<td>–</td>
</tr>
</tbody>
</table>

Chickens of all experimental groups were in the similar conditions and they were fed by standard mixed feed. The chicken states were assessed by the daily clinical observation. After 42-daily growing time six broilers were chosen from each group and live weight, mass of carcasse after killing were determined.

The obtained experimental data were statistically processed with the use of indicators of variation statistics of Microsoft Office Excel 2010. Student’s t-test and table values of student's criterion were used to assess the significance of differences. The differences were considered significant at p ≤ 0.05.

The results of the study and their discussion.

In previously conducted studies, it was found that the complexes of micronutrients increased the average daily weight gain by 2.0-6.0% (p≤0.05; p≤0.01) and the preservation by 4.0% [9, 10].

Blood analysis was conducted to evaluate the effects of new supplements on the health of the animals. The data are presented in table 2.

Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td>76,0±3,21</td>
</tr>
<tr>
<td>Erythrocytes, 10^{12}/l</td>
<td>2,1±0,04</td>
</tr>
<tr>
<td>Leucocytes, 10^{3}/l</td>
<td>39,1±3,01</td>
</tr>
<tr>
<td>Total protein, g/l</td>
<td>29,9±1,26</td>
</tr>
<tr>
<td>Albumin, g/l % to the total protein</td>
<td>17,7±0,91</td>
</tr>
<tr>
<td>Globulins, g/l % to the total protein</td>
<td>12,2±0,3</td>
</tr>
<tr>
<td>Albumin-globulin ratio</td>
<td>1,45</td>
</tr>
</tbody>
</table>

Note: * p≤0,05; ** p≤0,01
In the end of the experiment it was indicated a rising trend in hemoglobin contents and red blood cell count by 6.1-7.5% (p>0.05) in all experimental groups. White blood count had a rising trend only in the groups where malates were used and it was in the frame of the physiologically normal state. In these experimental groups the concentration of total protein in blood serum of chickens was increased by 1.8-12.4% (p>0.05). Decreasing of the concentration of albumins and increasing of the proportion of globulins in the blood by 32.3-37.7% (p≤0.01) were observed in the blood of chickens which consumed citrates. The proportion of albumins in the protein had a rising trend in the groups where chickens fed malates.

The data showed that the organic forms of biometals had the positive effect on the morphologic and biochemical blood composition. Malates had the advantage of citrates according to the changes in the proteinogram, albumin-globulin ratio and number of red blood cells.

At the external examination of carcasses was not detected any significant differences among the groups. Carcasses were yellowish color, the surface of the skin was dry, subcutaneous fat was yellowish tinge. The consistency of the organs was elastic and their cutting edges were not separated. Pectoralis was white with a pinkish tinge and elastic [11]. Broilers from the first (control) group had the lowest body weight. The differences of the control among the experimental groups (except the fourth group) and control were confirmed statistically.

The results of the research confirmed statistically valid positive effect of the biocoordination complexes of trance elements on meat productivity (table 3).

Table 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>1</td>
</tr>
<tr>
<td>±16.0</td>
<td>±17.0**</td>
</tr>
<tr>
<td>Mass of the eviscerated carcasses, g</td>
<td>1335</td>
</tr>
<tr>
<td>±14,5</td>
<td>±15.9*</td>
</tr>
<tr>
<td>Mass of muscles, g</td>
<td>639,0</td>
</tr>
<tr>
<td>±9,14</td>
<td>±8.5*</td>
</tr>
<tr>
<td>Mass of bones, g</td>
<td>312.1±5,3</td>
</tr>
<tr>
<td>Note: * p≤0.05; ** p≤0.01</td>
<td></td>
</tr>
</tbody>
</table>

It is known that liveweight gain of broilers is happen due to the unique of biosynthesis muscle proteins. We point out that the substitution of inorganic salts of iron, manganese and zinc on their chelate forms promoted the increasing the muscle mass.

Preslaughter weight in the groups was distributed in decreasing order of body weight as follows: second-fifth-sixth-third-seventh-fourth. Body weight of chicken fed malates has changed depending on doses: in the variant with the minimal dose it was higher than in the variant with middle and maximal concentration. Similar dependence was identified in the case with citrates. The given differences are statistically significant. The study found a positive correlation between the mass of the eviscerated carcasses and preslaughter weight. However there was a little difference in slaughter yield among the control and experimental groups. It demonstrates that experimental trance elements did not disturb the normal relation among somatic and visceral parts of the body.

At the end of the study muscle mass by using malates in diet was higher than in the control group by 45 g. per carcass (on average by 7.1%); when using citrates the parameter was only 4.9%. Mass of bones was changed slightly and it didn’t have statistically significant differences.

**Conclusion.**

Thus, the investigation of whole blood and its serum confirmed not only the safety of the new supplements for the health of birds, but also the positive effect of the new agents on the biochemical functions of the body. The used metal organic biometal complexes with malic and citric acids stimulated growth of broilers more effectively than their inorganic compounds.

More intensive increase of the body mass of the chickens can be attributed to the fact that trance elements are absorbed in the intestine easily and
quickly from organic compounds and they are transported on the specific enzymes for them, that determine the intensity of metabolic processes in the growing organism.

In previously conducted studies [9] it was showed that the content of the proteins and the proportion of tryptophan in protein were increased, the ratio of this amino acid and oxyprolin is the qualitative indicator of meat.

Designed supplements can be used for animal microelementosis prophylactic and treatment and also for stimulation of the productivity.

References
INFLUENCE OF L-LYSINE SULFATE ON CONTAINING OF VITAMINS AND MINERALS IN THE BODY OF BROILER CHICKENS

Abstract. Experimental researches have shown that the L-lysine sulfate (the product of microbiological synthesis) has a positive effect on metabolism of vitamins A, E, C in the liver and minerals calcium and phosphorus in the blood and bones of broiler chickens. The dose of L-lysine sulfate of 800 mg∙kg⁻¹ and 1000 mg∙kg⁻¹ weight body has influence on increasing vitamins level in the liver. Was observed, that indexes of calcium and phosphorus are increased in the blood but it’s decreased in the bones of broiler chickens. This results are necessary for Veterinary Pharmacology Consults and official practically using in agricultural sectors of Russian Federation.

Key words: L-lysine sulfate, broiler chickens, blood, liver, bones, concentration of vitamins A, E, C, calcium and phosphorus.

Introduction. L-Lysine Sulfate – feed additive used for enrichment and balance the rations of agricultural animals. Along with lysine in a preparation there are other substances that enhance its nutritional value: carbohydrates, mineral salts, organic acids, more than 10% of other amino acids [1]. L-Lysine Sulfate has greater exchange energy than lysine in hydrochloride form. The use of L-Lysine Sulfate in mixed feed production is economically viable and environmentally sound, and increase economic efficiency of production [3].

Vitamins and minerals play important role in the metabolism, growth and health in the body of animals. Vitamin A has multiple functions: it is important for growth and development, for the maintenance of the immune system and good vision [3]. Vitamin A is needed by the retina of the eye in the form of retinal, which combines with protein ops in to form rhodopsin, the light-absorbing molecule necessary for both low-light (scotopic vision) and color vision [5].

Vitamins E and C have antioxidant functions. Vitamin C acts as an electron donor for important enzymes [6], acting to lessen oxidative stress and an enzyme cofactor for the biosynthesis of many important biochemical [7]. As antioxidant, vitamin E acts in cell membranes where prevents the propagation of free radical reactions, although it has been also shown to have pro-oxidant activity. Non-radical oxidation products are formed by the reaction between alpha-tocopheryl radical and other free radicals, which are conjugated to gluconic acid and excreted through the bile or urine. Vitamin E is transported in plasma lipoproteins. After its intestinal absorption vitamin E is packaged into chylomicrons, which along the lymphatic pathway are secreted into the systemic circulation [8].

Minerals perform four broad types of function in animals: structural, physiological, catalytic and regulatory. One of the most important microelements are calcium and phosphorus. It can form structural components of body organs and tissues (bones, teeth and cartilages) [9].

Minerals and vitamins are usually transported from the serosal side of the mucosa to the liver in free or bound forms via the portal blood stream, but they can get ‘stuck’ in the mucosa. From the liver, they are
transported by the peripheral bloodstream to be taken up by different organs and tissues at rates determined by local transporter mechanisms in cell membranes and organelles to meet intracellular needs. [10].

Minerals follow labyrinthine pathways through the animal once ingested and Fig. 1.1 gives the barest of introductions. The entrance of some amino acids can enhance or constrain the proportions of ingested minerals that are absorbed from the diet and occasionally change the forms in which they are absorbed [10, 11].

Metabolism of calcium and phosphorus binds with processes of protein synthesis in the body as increasing mass muscles. Some experiments show, that chickens are able to adapt to early dietary changes in P and Ca through improvement of digestive efficiency in a later phase, and the extent of the compensation in terms of growth performance and bone mineralization depends on the P and Ca levels in the subsequent diet [12].

Thus, amino acid diet can influence on changes concentration minerals and vitamins in different organs and tissues in the organism broiler chickens.

The aim of this study was evaluate the capacity concentrations vitamins A,E,C in the liver and P and Ca in the blood and bones of broiler chickens with using diet from L-lysine sulfate.

**Methods and materials of research.**

The experiments were on the broiler chickens of cross "Hubbard" in period age since 1 before 39 days. All animals was separated on five groups by 10 individuals in each. Birds of control and experimental groups had diet from main ration (MR) with balanced nutritionally and biologically active substances feed. Chickens experimental groups along with the basic diet received a daily dose of lysine sulfate in accordance with Table. 1. Daily deliveryand delivery of feed to chickens were carried out according to the recommendations of the manufacturer of cross-country "Hubbard".

**Figure 1.** An illustration of ways flows of mineral between pools calcium and phosphorus in animal body (Braithwaite, 1983) [10].

**Table 1.**

<table>
<thead>
<tr>
<th>Groups of broiler chicken</th>
<th>Number of broiler chicken</th>
<th>Scheme of feeding</th>
<th>Dose of L-lysine sulfate, mg·kg⁻¹·weight body</th>
<th>Frequency of feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>10</td>
<td>main ration (MR)</td>
<td></td>
<td>every day</td>
</tr>
<tr>
<td>II (experimental)</td>
<td>10</td>
<td>MR + lysine sulfate</td>
<td>700</td>
<td>every day</td>
</tr>
<tr>
<td>III (experimental)</td>
<td>10</td>
<td>MR + lysine sulfate</td>
<td>800</td>
<td>every day</td>
</tr>
<tr>
<td>IV (experimental)</td>
<td>10</td>
<td>MR + lysine sulfate</td>
<td>900</td>
<td>every day</td>
</tr>
<tr>
<td>V (experimental)</td>
<td>10</td>
<td>MR + lysine sulfate</td>
<td>1000</td>
<td>every day</td>
</tr>
</tbody>
</table>

It was organized by the outdoor maintenance of broiler chickens with free access to food and water. Light, temperature and other climate parameters comply with the established veterinary standards and norms [13]. Temperature, lighting and ventilation was held in the room where the birds were kept. A soft bed of fresh dry sawdust was changed every five days or added as needed. Daily weighing of feed was conducted in accordance with the biological bird demand for nutrients. Live weight of chickens was measured to an accuracy of 1 g to morning feeding every five days during the period of experience.
At the end of the experience produced poultry slaughtered by decapitation and took tissue samples and organs for laboratory testing. Vitamins A and E were determined with method of spectral analysis; vitamin C – titrimetric method with using solution of 2,6-dichlorophenol.

Calcium was research with trilonometric method in the bones and titrimetric by de Waard in the blood. Phosphorus was determined with method of colorimetric by vanadate-molybdenum reagent in the bones and in the blood.

The resulting material was digitally processed using mathematical methods of mathematical statistics, taken in Biology and Medicine (Microsoft Excel 2007 application).

**Results and discussion.**

Indexes of the vitamins A, E, C was found in the liver of broiler chickens. Results of testing are shown in the table 2.

<table>
<thead>
<tr>
<th>Index</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (control)</td>
</tr>
<tr>
<td>Vitamin A,</td>
<td>82,22±1,16</td>
</tr>
<tr>
<td>mg/kg</td>
<td></td>
</tr>
<tr>
<td>Vitamin E,</td>
<td>236,40±4,37</td>
</tr>
<tr>
<td>mg/kg</td>
<td></td>
</tr>
<tr>
<td>Vitamin C,</td>
<td>281,60±8,97</td>
</tr>
<tr>
<td>mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

* reliably difference between control and experimental index, p<0,05

The heightened level of vitamin A was in the liver of animals which take diet from MR and lysine sulfate in dose of lysine sulfate 800 and 1000 mg·kg⁻¹ weight body. Concentration of vitamin C is increased with dose of lysine sulfate more than 800 mg·kg⁻¹ weight body. Index of vitamin E was reliably higher in third group in contrast with control.

As far as, ration of chickens did not contain other sources of vitamins, that we extrapolate positive effect entering lysine sulfate in nutrition of broiler chickens.

Calcium and phosphorus are accumulated in the bones and it takes part in functioning of different biosynthesis process in the blood [9]. Therefore we described to check levels of these microelements in the blood and in the bones.

Indexes of calcium and phosphorus have trend to increaseconcentration of this microelements in accordance with dose of lysine sulfate in the blood of birds (fig. 2).
Change of concentrations calcium and phosphorus have trend to decrease this indexes in the bones of animals (fig. 3).

This aspect demonstrates as calcium and phosphorus increases in the blood in the reason of its extraction from the bones for future of protein synthesis.

Conclusions.

Using of lysine sulfate in daily ration broiler chickens make possible increase vitamin A on 17% in the liver in dose 800 mg·kg⁻¹ and 1000 mg·kg⁻¹ weight body. Concentration of vitamin E can increased before 30% with using lysine in diet. The level of vitamin C also was heightened in dose 800 mg·kg⁻¹ weight body. Economic profitably use L-lysine sulfate in dose 800 mg·kg⁻¹ weight body.

Trend indexes of calcium and phosphorus are increased in the blood but it’s decreased in the bones of broiler chickens with entering L-lysine sulfate. Calcium/phosphorus ratio is demonstrated in physiologically norm.

Reference


PHARMACOLOGYAL REVIEWS

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DEVELOPMENT PERSPECTIVES OF NEW GENERATION MEDICATIONS BASED ON THE REDOX SYSTEM REGULATORS

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Abstract. This survey paper describes the necessity of the development of new medications influencing the body redox-potential. It supports the most pressing branch of pharmacology, which coincides with logically relevant attempts to shift paradigm of pharmacology from molecular to electronic, quantum-wave. This article covers and logically sorts research results of recent years and opinions of a wide range of scientists from various countries. The authors also give their own assessment of the possibility to influence body redox potential. It is reported that some biophysical achievements regarded undoubtedly put a new spin in pharmacology of the biophysical level. These research results devoted to the role of redox-potential in regulation of biological systems are considered to open up new opportunities for pharmacotherapy of pathological conditions by developing medications of the new generation – redox-potential regulators - aimed at the induction of the body protective resources. The paper further reports that elaborate study of redox-potential in providing biological systems regulation has resulted in the detailed investigation of Mexidol benefits. Special attention is paid to the general principle of action of endogenous redox regulators: metabolic, energetic and informational. The article also highlights key issues of the regulation of oxidation-reduction processes in the body and, consequently, the role of reactive oxygen species in physiology and pathology. The paper reasonably concludes with the statement on the necessity to turn pharmacologists’ attention not only to improving the existing antioxidant preparations, but to developing the redox system regulators, which appear to be medications of the new generation for pathogenic therapy.

Key words: reactive oxygen species, oxygen, reduction-oxidation reactions, redox potential, pharmacotherapy.

Introduction. Use of oxygen to receive energy needed for the life-sustaining activity by substrate oxidation in biological objects is considered to be one of the mainstems of the living systems evolution [1]. Functioning of the human body as the most highly organized life-form depends on oxygen supply; its deficiency causes multiple pathological conditions. Decrease of energy resources under hypoxia results in multisystemic and multiorganic functional-metabolic changes and, subsequently, death of the whole body.

Oxygen is the leading factor of the biological rhythms control: circadian, seasonal, reproductive, as well as homeostasis, proliferation, differentiation, apoptosis, carcinogenesis, aging, necrosis and some other cellular processes [2-5], and their exact mechanisms can be understood only in the plant cell [6].

Aerobic organisms have oxidative potential, which increases with the increase of tissue blood supply or activation of free-radical particles. Oxygen deficiency, on the contrary, leads to reductive change of potential. Shift of the balance between prooxidants...
and anti-oxidants induces oxidative stress characterized by specific changes of the cellular processes: membrane structure is violated due to lipid peroxidation, proteins are oxidated, DNA is damaged, cellular redox potential changes.

There have been recorded instances of more than 200 nosological forms accompanied by oxidative stress: cardio-vascular [7-14], oncological [15], infectious diseases [16, 17], pathologies of the respiratory [18], reproductive [19-21], urinary [22, 23], nervous system, pyoinflammatory processes [24], arthritis of various types, diabetes mellitus, cataract and others [25].

Mitochondrial respiratory chain, microsome electron-transport chain, arachidonic acid metabolism, hypoxanthine-xanthine oxidase reactions, biosynthesis and catecolamine oxidation are reported to be sources of reactive oxygen species (ROS) [26]. Mitochondriums are stated to be the main sources of radicals in a cell [27], therefore these organelles need to be constantly protected from damages induced by oxidative stress. Mitochondrial DNA appears to be the most vulnerable target for ROS. There exist a lot of evidences that its oxidative violation and increasing de-energization of oxygen-dependant cells play the key role in the whole range of “mitochondrial diseases” [28, 29], neurodegenerative pathologies [30, 31], as well as body aging [32]. The final stage of this process is necrosis, due to which a cell as a living system stops existing, since transformation systems of substance and energy flows, permeability of cellular and subcellular membranes are violated, ion gradients disappear [1]. Currently, the role of mitochondriums in regulation of proliferation and differentiation processes, apoptosis and cancerous growth is actively investigated. Change of ROS generation rate under influence of mitochondriums may be considered as one of the mechanisms that switch functional activity of a cell [33].

ROS comprises a lot of transitional products of oxygen metabolism producing in the body; they, in turn, having high reactive capacity can lead to violation of practically all structural components of the biological system [2, 33-36]. Increased ROS levels result in initiation of free-radical oxidation – the process of immediate oxygen transfer on substrate with formation of peroxides, ketones, aldehydes inducing reactions of peroxide oxidation. This ancient natural destructive mechanism that has a hold over all the organic compounds is necessary for the following renovation of cells and tissues, their adaptation for the changing environment, body protection against infections, participation in formation of biologically active compounds. This implies the fact that presence of free radicals in a body has specific, physiologically important significance [37].

On the other hand, it is beyond argument that functional properties of some enzymes, carbohydrates, proteins, DNA and RNA are changed under the influence of free-radicals so that a cell loses its regulatory functions [2]. Concurrently there may appear abnormal proteins; secondary destructive processes may be stimulated apart from direct damaging action.

Discussion. The leading role in pathogenesis of radiation damage, inflammatory processes of various localization and origin, development of hyper- and hypoxic conditions, post-ischemic, reperfusion and hyperoxic disorders, wound processes, stress, acute and chronic hepatic diseases, myocardial infarction, strokes, atherosclerosis, carcinogenesis, aging and so on belongs to lipid peroxidation. Influence of free-radicals on structure and functions of biological membranes is one of the most important pathogenic mechanisms in hypoxia.

There is a multilevel physiological system of protection against oxidation agents in a body – the antioxidant system supporting oxidative-antioxidative balance in all organs and systems [38, 39]. The antioxidant system includes the whole complex of enzymes: superoxide dismutase (SOD), catalase, glutathione-dependant peroxidases, transferases etc., as well as a range of cellular metabolites: lipoic, ascorbic, uric acids, tocopherols, carotenoids, flavonoids, polyphenols, carnosine, bilirubin, coenzyme Q10 and other compounds aimed at maintaining the normal reactions of the body in various pathological conditions, including hypoxia [2, 32, 40-50]. Malfunctioning of these systems is stated to be one of the most significant factors in violation of prooxidant-antioxidant balance and oxidative stress development [51, 52]. Some research studies have demonstrated that ROS formation and multicomponent anti-oxidative protection constitute the unified system being in the dynamic balance and having capacity for self-regulation [35]. Due to the associated functioning of the ROS generation and anti-oxidative protection systems oxidation-reduction balance is established in a cell, in other words redox status. Redox potential formation reflecting balance status of the pro- and antioxidant body systems [53] is greatly influenced by protein components of blood plasma, catalase enzyme activity and, possibly, alpha-synuclein level [54].
The importance to maintain such balance for the living system was marked by A. Szent-Györgyi [55] in the mid-XXth century; he considered the balance between electron donors and acceptors to be one of the basic life parameters.

Associated redox system representing combination of redox cycles of carbon, nitrogen, oxygen and sulphur, has been formed during the evolution process [56]. Redox potential of the normally functioning cells is maintained at the constant level and changes only under specific actions [1, 34, 57-59]. In spite of the fact that oxidation-reduction reactions are the basic reactions of the bioenergetic processes and regulate cellular activity as a whole [27], there is no clear understanding of their role in the intra- and extracellular pathogenesis, signaling processes, homeostasis regulation. However, redox homeostasis acquires conceptual significance in some pathological body processes [60].

Redox potential reduction shift forms the basis of triggering hypoxic necrobiosis and damaging specific oxygen-sensitive cells; redox potential oxidation shift forms the basis of developing free-radical necrobiosis and apoptosis of trophic cells [1]. Oxidative and nitrosative stress removes SH-SS balance towards oxidated thiols that causes neurons necrosis and death [61].

Insignificant redox-potential shifts through a biological cycle give an opportunity to rhythmically regulate phases of functional activity of the living systems with resting phases; and its significant shifts lead to activation of cellular death processes. Thus, for example, correlation of reductive and oxidated glutathione levels plays role of a special “switch” from proliferation phase to differentiation phase and further to apoptosis [62].

Redox potential shift has an effect on realization of metabolic processes, work of the signaling transduction system, gene expression, activity of transcription factors [63-66], change of activity and biological full value of both intracellular compartments and a cell as a whole [67]. When changing redox potential status cellular strategy is mostly defined by the status of signal transmitting systems [68]. Redox potential has been demonstrated to be an indicator of cellular functional activity and effectiveness of anti-oxidated protection [69].

Organic substances (vitamins, aminoacids) and compounds of inorganic origin — macro- and microelements - take part in redox potential regulation alongside with oxygen; even insignificant change of concentration of inorganic substances has an effect on the functioning of the whole body [1, 70].

The data obtained on the leading role of redox potential in maintaining the biological system regulation give new opportunities for pharmacotherapy of pathological conditions including hypoxic one. Currently the amount of theoretical, experimental and clinical data is sufficient to create a theory of developing highly effective medications of new generation — redox regulators — based on the study of functional biological multivaluedness of redox-active agents.

The enormous list of modern preparations for chemotherapy is represented by chemical compounds foreign for the biological system, having xenobiotic load on a body and, consequently, causing side effects. Therapeutical effect of any xenobiotic is restricted by the blockage of adaptational body reactions to a damage and replacement of the proper protective resource into the artificial one until its atrophy. Besides, introduction of foreign medications can result in resistance that increases danger of overdose and individual hypersensitivity. Severe drawbacks of modern medications are reported to be a narrow range of pharmacological activity and absence of selectivity towards a biotarget; that is why a positive result in vivo for a foreign compound is accidental; this fact is proved by rare successful cases of numerous chemicals screening.

Synthesis of effective and safe medications – natural participants of enzymatic reactions and metabolism compatible with biological structures and systems, - is possible by applying electrophilic replacement, redox vitamin modification and complex formation. The example of redox vitamin modification appears to be an effective polyfunctional preparation – mexidol – vitamin B₆ modification (redox oxypyridine active centre) with succinate [71].

Analysis of numerous investigations of this medication allows concluding that vitamin B₆ easily entering a cell through its own natural canals transfers a succinate; this makes mexidol an irreplaceable pathogenetic preparation in complex therapy of post-hypoxic conditions [72, 73]. Vitamin B₆ as an active redox agent participates in the regulation of oxidation-reduction processes in cytosol and membrane, and a succinate metabolite – succinic acid – reactivates cell respiration. Due to such synchronic tandem cells living activity remains unchanged and a chance to restore cellular functional activity under oxygen deficiency increases.

Impact of succinate on the energy exchange has been studied more in details for all substrates of the Krebs cycle [74]. Predominant use of succinate is natural cell protection against hypoxia. At that,
replenishment of the substrate fund may occur as a result of the Krebs cycle reactions, both – linear and reverse. Such a peculiarity of oxidative phosphorylation provides an opportunity to reverse reaction at the di-carbon stage of the Krebs cycle with transition of fumarate into succinate and increasing amount of the latter. Mechanism of invasive fumarate transformations during the Krebs cycle explains the efficient use of fumarate-containing preparation – sodium fumarate, mafusol, polyoxifumarin, konfumin, as well as the complex fumarate + Hydroxyethylstarch, which is successfully passing through the last stage of pre-clinical trials [75, 76]. Preparations of this group have come to stay in the program of fumarate-containing solutions for infusions applied in Accident and Emergency Departments of healthcare facilities in the Russian Federation and CIS-states when delivering medical care to the injured in military conflicts, natural and technological disasters [77].

Currently there has been shown an opportunity of redox potential pharmaco-correction of heart failure caused by ischemic heart disease with adenosine containing reduced NAD form [78]; there have been revealed pre-conditions for Histochrom application in complex therapy of venous retinal occlusions associated with changes of the redox system [79].

Redox regulators take the exceptional position among biomolecules, because only redox-active substances are able to transfer electrons intermolecularly. Since charge redistribution is considered to be a basis of a biochemical reaction, then only redox-active elements and molecules specific for the given enzyme play the functional role. This explains synchronization of biological processes occurring at all levels of the systemic organization influenced by associated redox-factors. At that, the significance of the unique organization of a protein molecule or its active polypeptide part is evident.

An attempt to change redox potential of the body liquid media with ionized liquid having either positive or negative potential has been made in the Voronezh N.N.Burdenko State Medical University [80, 83]. Some research studies performed prove safe application of fluids with various redox potential, establish therapeutic range of the redox potential parameter in millivolts [81, 82].

Variety of biological systems, numerosity of active particles and secondary messengers, complexity of the inflammatory reaction mechanism and variety of factors influencing it do not allow creating a precise picture of mechanisms, which realize protective potential of safe medications. However, universe character of their participation in these mechanisms as a redox potential agent supports not only reasonability but also the necessity of creating such agents.

Redox agents perform an antioxidant function as constituents of the physiological antioxidant system, however, the fact of even greater importance is that they provide functional enzymes activity as co-enzymes, co-substrates and co-factors, i.e. reveal prooxidant activity. More than that, redox agent activity is defined by the redox environment, mainly, protein having signaling transduction.

General principle of endogenic redox regulators impact is aimed at three biological flows: metabolic, energetic and informational; their synchronic interaction at all levels of organization of the living system is achieved due to redox factors incorporation into enzymatic processes. Simultaneously they act as sensors responding to changes of one of the most important indicators of the biological system – redox potential. A received signal is directly or indirectly transformed into activity shift of a specific enzyme.

Currently redox-sensitive elements of the intracellular signal-transmitting systems (p38 MAP kinase, INK, transcription factors and proteins of Bcl-2 family) are shown to be molecular targets for therapeutic correction of the apoptotic program violation under oxidative stress [68].

In the extreme (pathological) situation associated with hypoxia anti-oxidants are able to reveal their own protective function acting as an electron buffer. Redox-active agents, on the contrary, produce induction and mobilization of all protective resources at any level of the biological system demonstrating not only anti-oxidant, but pro-oxidant activity as well. The problem to be studied is how to trigger these processes and further regulate them.

If a danger of stress-reaction is insignificant and the amount of operative protective resource is sufficient enough to eliminate its consequences, then participation of endogenic redox regulators may be considered as manifestation of their preventive potential.

Different situation occurs if the amount of protective resource is insufficient and it is necessary to mobilize specific protective proteins, information on which is kept in the genetic material. It is the medication based on modified signaling molecules of proteins that should participate in the mechanisms of induction and mobilization of protective resource. Such varieties of redox-active signaling molecules performing the role of the protective function trigger may claim to be highly effective medications.
Development of organic complexes of biometals seems to be the most universal. In this case organic bioligand being a natural redox-active agent of an enzyme allows an element to be the most effectively included in the metabolic mechanism. Such complex compounds of transitional biometals may reproduce chemical behaviour of metallo-enzymes in a cell. The role of these complexes is restricted by the participation of electrons and redox-reactions typical for this enzyme in the processes of transport. Low-molecular compounds with metals, for example, zink sulfate or gluconate, cannot be referred to the category of redox regulators, since absence of a relevant ligand in their composition deprives these compounds of substrate specificity. Taking into account high level of biological activity of essential bioelements their reliable and fast delivery by transport proteins with easier release appears to be a warrant of high effect.

**Conclusion.** Thus, any violation of homeostasis results in pathological conditions causing violation of energy production, storage and utilization. Redox-potential is considered to be the basic indicator of metabolic cell status integrating uncountable number of oxidation-reduction reactions. The data available on the leading role of redox-potential changes in providing the biological systems regulation give new opportunities for pharmacotherapy of pathological conditions including hypoxic ones. Development of medications – redox system regulators – aimed at the induction of proper protective body resource appears to be strategic concern of creating new generation pathogenic therapy agents.

**References**

1. Shilov V.N. Molecular mechanisms of structural homeostasis (Moscow, «Intersignal», 2006), 288 p. (In Russian) [eLIBRARY]
RESEARCH RESULT:

PHARMACOLOGY AND CLINICAL PHARMACOLOGY


32. Martinovich G.G., Cherenkevich S.N. Oxidation-reduction processes in cells. (Minsk: BGU, 2008), 159 p. (In Russian) [eLIBRARY] [Full text]


34. Zenkov N.K., Lankin V.Z., Men'shikhova E.B. Oxidative stress: biochemical and pathophysiological aspects. (Moscow: Nauka, Interperiodika, 2001), 343 p. (In Russian) [eLIBRARY]


36. Ulashchik V.S. Active oxygen forms, antioxidants and therapeutic physical factors effect. *Issues of Resort Medicine, Physiotherapy and Physical Therapy*. 2013, Nø 1, pp. 60-69. (In Russian) [eLIBRARY]


38. Statisenko M.E., Turkina S.V., Kosivtsova M.A. Opportunities of mexicor when using it as a constituent of the combined therapy in patients with ischemic heart disease and diabetes type II. *Clinical medicine*. 2013, Nø 5, pp. 59-64. (In Russian) [eLIBRARY]


44. Hong Z., Hailing L., Hui M., Guijie Z. Effect of vitamin E supplementation on develop-ment of


66. Goroncharovskaya I.V., Makarov M.S., Kolesnikov V.A. Redox potential as a characteristic feature of thrombocytes living activity. Achievements in Chemistry and Chemical Technologies. 2015, № 3(162), pp. 35-37. (In Russian) [Full text]


70. Voronina T.A. Mexitol: range of pharmacological effects. Journal of Neurology and Psychiatries. 2012, №12, pp. 86–90. [eLIBRARY] [Full text]


72. Schul'kin A.V. Influence of mexitol on the development of neurons excitotoxicity phenomenon in


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